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☐	L12	biocarb.asn.	34
☐	L11	0098252	32
☐	L10	8604065	13
☐	L9	teneberg.in. and karlsson.in. and natunen.in.	18
☐	L8	2002056893	3
☐	L7	056893	0
☐	L6	02056893	2
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=OR	
☐	L5	L4 same (saccharide or oligosaccharide or oligo-saccharide)	9
☐	L4	helicobacter near25 (antagonist or inhibitor or blocker)	543
		DB=USPT; PLUR=YES; OP=OR	
☐	L3	L2 same oligo$	7
☐	L2	helicobacter near25 (antagonist or inhibitor or blocker)	145
☐	L1	heliocbacter near25 (antagonist or inhibitor or blocker)	0

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☐	L11	0098252	32
☐	L10	8604065	13
☐	L9	teneberg.in. and karlsson.in. and natunen.in.	18
☐	L8	2002056893	3
☐	L7	056893	0
☐	L6	02056893	2
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=OR	
☐	L5	L4 same (saccharide or oligosaccharide or oligo-saccharide)	9
☐	L4	helicobacter near25 (antagonist or inhibitor or blocker)	543
		DB=USPT; PLUR=YES; OP=OR	
☐	L3	L2 same oligo$	7
☐	L2	helicobacter near25 (antagonist or inhibitor or blocker)	145
☐	L1	heliocbacter near25 (antagonist or inhibitor or blocker)	0

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not to be considered

L5: Entry 2 of 9 File: PGPB Sep 9, 2004

DOCUMENT-IDENTIFIER: US 20040176320 A1
TITLE: Use of at least one glycoinhibitor substance

Summary of Invention Paragraph:
[0009] US patents of Zopf et al.: U.S. Pat. No. 5,883,079 (March 1999), U.S. Pat. No. 5,753,630 (May 1998) and U.S. Pat. No. 5,514,660 (May, 1996) describe Neu5Ac.alpha.3Gal-containing compounds as inhibitors H. pylori adhesion Sialyl-lactose molecule inhibits Helicobacter pylori binding to human gastrointestinal cell lines (Simon et al., 1999) and is also effective in an rhesus monkey animal model of the infection (Mysore et al., 2000). The compound is in clinical trials. The present invention is not related to use of antiadhesive oligosaccharides.

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(74) Agent: **OY JALO ANT-WUORINEN AB**; Iso Roobertinkatu 4-6 A, FIN-00120 Helsinki (FI).

(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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13 November 2003

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/056893 A1

(54) Title: NOVEL RECEPTORS FOR $I(HELICOBACTER PYLORI) AND USE THEREOF

(57) Abstract: The present invention describes a substance or a receptor comprising *Helicobacter pylori* binding oligosaccharide sequence $[Gal(A)_q(NAc)_r/Glc(A)_q(NAc)_r\alpha 3/\beta 3]_s[Gal\beta 4GlcNAc\beta 3]_tGal\beta 4Glc(NAc)_u$ wherein q, r, s, t, and u are each independently 0 or 1, and the use thereof in, e.g., pharmaceutical and nutritional compositions for the treatment of conditions due to the presence of *Helicobacter pylori*. The invention is also directed to the use of the receptor for diagnostics of *Helicobacter pylori*.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI 2003/000039

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 02056893 A1	25.07.2002	18.01.2002	19.01.2001

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure (day/month/year)	Date of written disclosure referring to non-written disclosure (day/month/year)
STN International, File CAPLUS, CAPLUS accession no. 2002:390032, document no. 138:1587, Chandrasekaran, E. et al, "Biosynthesis of the carbohydrate antigenic determinants, Globo E, blood group H, and Lewis b: a role for prostate cancer cell alpha1,2-L-fucosyltransferase"& Glycobiology (2002), 12(3), 153-162		

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI 2003/000039

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

In the summary of the invention (page 3 of the description) is explained that "y is either alpha or beta indicating the anomeric structure of the terminal monosaccharide residue". Accordingly, Hex1 in claim 1, $Glc(A)_{q1}(NAc)_{r1}\beta3$ in claim 4, $Glc\beta3$ in claims 9-10, $GlcNAc\beta3$ in claim 10, $Glc(A)_q(NAc)_r\alpha3/\beta3$ in claim 38 and $Gal(A)_q(NAc)_r/Glc(A)_q(NAc)_r\alpha3/\beta3$ in claim 41 are intended to be terminal monosaccharide residues. This seems to be a relevant feature of the invention and should therefore be indicated in said independent claims. The present claims 1, 4, 9-10, 38, 41 and the respective dependent claims include non-terminal sequences.

Table 1 (continued)

20	NeuAcα6-SPG	NeuAcα6Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	HM	(Nilsson *et al.*, 1981)
21		Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	(+)	RT[d]	(Miller-Podraza *et al.*, 2001)
22	A6-2	GalNAcα3(Fucα2)Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	HE	(Laine *et al.*, 1974)
23	B6-2	Galα3(Fucα2)Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	HE	(Koscielak *et al.*, 1973)
24	NeuAc-x_2	NeuAcα3GalNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	HE	(Watanabe and Hakomori, 1979)
25		GalNAcβ3Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	RCC	(Thurin *et al.*, 1989)
26		Galβ4GlcNAcβ6(Galβ4GlcNAcβ3)Galβ4Glcβ1Cer	-	BB	(Teneberg *et al.*, 1994)
27		NeuGcα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	(+)	RT	(Lanne *et al.*, 2001)
28		Galα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	RT	(Lanne *et al.*, 2001)
29	A7-2	GalNAcα3(Fucα2)Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	-	DSI	(Falk *et al.*, 1979c)
30	B7-2	Galα3(Fucα2)Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	-	HE	

Footnotes to Table 1

a The glycosphingolipid shorthand nomenclature follows recent recommendations (Nomenclature of glycoproteins, 1988).

b The following abbreviations are used for the glycosphingolipid sources: RT, rabbit thymus; HE, human erythrocytes; RE, rabbit erythrocytes; HM, human meconium; RCC, rat colon carcinoma; BB, bovine buttermilk; DSI, dog small intestine.

c Definition of binding strength is as follows: + denotes a significant darkening of the autoradiogram with 4 μg applied on the TLC plate, (+) indicates a weak to intermediate darkening while a minus sign signifies no binding.

INTERNATIONAL SEARCH REPORT

Intern[illegible] application No.
PCT/FI 03/00039

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File CAPLUS, CAPLUS accession no. 2001:118779, document no. 134:292940, Fujita, M. et al, "Ancorinosides B-D, inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP), from the marine sponge Penares sollasi Thiele", & Tetrahedron, (2001), 57(7), 1229-1234	1,17,19-21, 24-25,35,65
X	STN International, File CAPLUS, CAPLUS accession no. 1998: 534525, document no. 129:258636, Miura, Yoshiaki et al, "alpha-N-Acetylgalactosamine-capping of chondroitin sulfate core region oligosaccharides primed on xylosides", & Glycobiology (1998), 8(8), 813-819	1-2,4,7,10, 12-13,20-21, 24-25,35,65
X	STN International, File CAPLUS, CAPLUS accession no. 1994:455685, document no. 121:55685, Yamamoto, Kazuo et al, "Interaction of Immobilized Recombinant Mouse C-Type Macrophage Lectin with Glycopeptides and Oligosaccharides", & Biochemistry (1994), 33(26), 8159-66	1,13,20-21, 24-25,35-65
X	STN International, File CAPLUS, CAPLUS accession no. 1989:572588, document no. 111:172588, Kratchanov, C. et al, "Reaction of apple pectin with ammonia", & International Journal of Food Science and Technology (1989), 24(3) 261-7	60-64
X	STN International, File CAPLUS, CAPLUS accession no. 1989:171879, document no. 110:171879, Anger H. et al, "Amidated pectins - characterization and enzymic degradation", & Food Hydrocolloids (1988), 2(5), 371-9	60-64
P,X	WO 02056893 A1 (CARBION OY), 25 July 2002 (25.07.02)	1-55,60-65

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 03/00039

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	STN International, File CAPLUS, CAPLUS accession no. 2002:390032, document no. 138:1587, Chandrasekaran, E. et al, "Biosynthesis of the carbohydrate antigenic determinants, Globo E, blood group H, and Lewis b: a role for prostate cancer cell alpha1,2-L-fucosyltransferase"& Glycobiology (2002), 12(3), 153-162	1,17,20-21, 24-25,35,65
A	WO 9827988 A1 (NUTRAMAX LABORATORIES), 2 July 1998 (02.07.98)	56-59
A	EP 0354595 A1 (UNILEVER PLC), 14 February 1990 (14.02.90)	56-59
A	US 3405120 A (TAKEHIKO KAWANO ET AL), 8 October 1968 (08.10.68)	56-59
A	STN International, File CAPLUS, CAPLUS accession no. 1990:512024, document no. 113:112024, Pepop. Rep. China, "Method of production of D-aminogalactose hydrochloride for clinical anlysis", & CN,A,1036386,19891018	56-59
A	STN International, File CAPLUS, CAPLUS accession no. 1967:450920, document no. 67:50920, Jeffrey, P.L. et al, "An improved method for the isolation of hexuronic acid from chondroitin sulfate preparations", & Biochimica et Biophysica Acta (1967), 141(1), 179-81	56-59

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 02/00043

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/702, C07H 15/04, C07H 3/06, A61P 1/04, A61P 31/04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, C07H, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, CHEM.ABS.DATA, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 0143751 A1 (A+ SCIENCE INVEST AB), 21 June 2001 (21.06.01) --	1,6-21, 23-24,26-33
X	Glycobiology, Volume 8, No. 4, 1998, Jonas Ångström et al: "The lactosylceramide binding specificity of Helicobacter pylori", pages 297-309 --	1-33
X	Infection and Immunity, June 1993, B.D. Gold et al: "Helicobacter mustelae and Helicobacter pylori Bind to Common Lipid Receptors in Vitro", pages 2632-2638 --	1-33

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 April 2002	25 -04- 2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer EVA JOHANSSON/BS Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 2004/000027

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07H 15/04, C07H 3/06, C07H 1/00, A61K 31/702, A61K 31/7028,, A61K 31/715, A61K 31/726, A61P 1/04 , A61P 1/16, 1/18, 9/00, 17/00, 31/04,35/00,37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07H, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CHEM.ABS.DATA, EPO-INTERNAL, WPI DATA, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02056893 A1 (BIOTIE THERAPIES CORP.), 25 July 2002 (25.07.2002) --	1-6,14, 17-52,54-60, 70,72
X	WO 03002128 A1 (CARBION OY), 9 January 2003 (09.01.2003) --	1-6,14, 17-52,54-60, 70,72
P,X	WO 03059924 A1 (BIOTIE THERAPIES OYJ), 24 July 2003 (24.07.2003) --	1-60, 70 AND 72

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2004	09 -07- 2004
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer PER RENSTRÖM/BS Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 02/00043

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Archives of Biochemistry and Biophysics, Volume 277, No. 1, February, 1990, Eric H. Holmes et al: "Characterization of Two Monoclonal Antibodies Specfic for Lacto-Series Type 1 Chain Galβ1-3GlcNAc-Terminal Structures", pages 181-188	1-33
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X	EP 0098252 B1 (BIOCARB AB), 11 January 1984 (11.01.84)	1-33
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X	WO 8604065 A1 (SYNTEK AB), 17 July 1986 (17.07.86)	1-33
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A	Glycoconjagate Journal, Volume 18, 2001, Maan Abul-Milh et al: "In vitro binding of Helicobacter Pylori to Monohexosylceramides", pages 253-260	1-33
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A	J. Biochem., Volume 108, 1990, Per Falk et al: "Bacteria of the Human Intestinal Microbiota Produce Glycosidases Specific for Lacto-Series Glycosphingolipids", pages 466-474	1-33
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A	Infection and Immunity, Volume 65, No. 6, June, 1997, Halina Miller-Podraza et al: "Binding of Helicobacter pylori to Sialic Acid-Containing Glycolipids of Various Origins Separated on Thin-Layer Chromatograms", pages 2480-2482	1-33
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A	Patent Abstracts of Japan, abstract of JP,A,10045602 (MURAKAMI MOTOYASU; KAKEN PHARMACEUT CO LTD), 17 February 1998 (17.02.98)	1-33
	--	
A	EP 0089938 A1 (SVENSKA SOCKERFABRIKS AB), 28 Sept 1983 (28.09.83)	1-33
	--	

Preferably, the reducing end monosaccharide is β-linked, when w is 0. In a preferred embodiment the reducing end monosaccharide structure is an open chain reducing end derivative of the monosaccharide unit. Preferably the structure is not linked to a ceramide, more preferably the structure is not linked to a ceramide comprising a hydroxyl fatty acid.

In a preferred embodiment w is 1 and the reducing end monosaccharide is not glycosidically conjugated to another monosaccharide unit, more preferably the reducing end monosaccharide unit is in an open chain reducing end derivative.
In a preferred embodiment A in the Formula is amide, methylamide or ethylamide of the carboxylic acid group of the glucuronic acid residue.
One embodiment of the present invention is a substance or a receptor binding to *Helicobacter pylori* comprising the oligosaccharide sequence according to separately preferred formulas:

Formula 2

$[Hex1(A)_{q1}(NAc)_{r1}y3]_sGal(NAc)_{r2}\beta4Glc(A)_{q2}(NAc)_{r3}\beta6Hex3(NAc)_{r5}$

or

Formula 3

$[Hex1(A)_{q1}(NAc)_{r1}y3]_sGal(NAc)_{r2}\beta4Glc(A)_{q2}(NAc)_{r3}\beta3Hex3(NAc)_{r5}$

or

Formula 4

$[Hex1(A)_{q1}(NAc)_{r1}y3]_sGal(NAc)_{r2}\beta4Glc(A)_{q2}(NAc)_{r3}\beta3Gal(NAc)_{r5}[\beta4Glc(A)_{q3}(NAc)_{r6}]_u$

wherein q1,q2,q3, r1, r2, r3,r5, r6, s, t, and u are each independently 0 or 1, and Hex1, and Hex3 are hexose structures, preferably mannose (Man), galactose (Gal) or glucose (Glc), and most preferably Gal or Glc, which may be further modified by A and/or NAc groups; y is either alpha or beta indicating the anomeric structure of the terminal monosaccharide residue with the provisions that at least one of the integers r2, q2, or q3 is 1 or r5 is 1 when u is 1, preferably r2 is 1 or q2 is 1, and analogs or derivatives of said oligosaccharide sequence having binding activity to *Helicobacter pylori* for binding or inhibiting *Helicobacter pylori*.

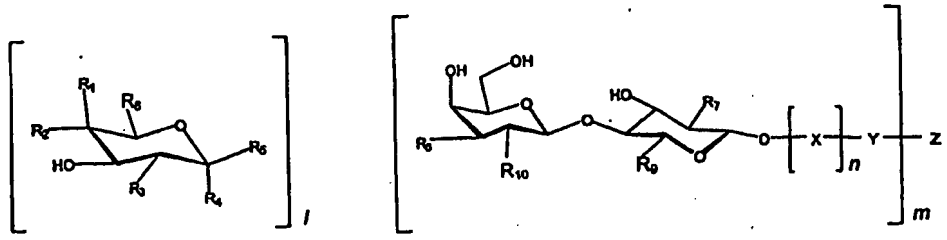
2. derivatization of carboxylic acid groups or 6-aldehydo groups or primary amine groups of the polymer to secondary or tertiary amines or to amides, when step 1 is applied, step 2 is optional.
3. hydrolysis of the polymer to corresponding monosaccharides.

The hydrolysis to monosaccharides may also be partial and produce useful disaccharide or oligosaccharide to produce analog substances. Preferably the hydrolysis produces at least 30 % of monosaccharides. Methods to produce the chemical steps are known in the art. For example oxidation of the polysaccharides to corresponding monoaccharides can be performed as described by Muzzarelli et al 1999 and 2002. These methods are preferred to the use of non-protected monosaccharides, because the protection or reactive reducing ends of the monosaccharides is avoided.

In a preferred embodiment the oligosaccharide sequences comprising GlcAβ3Lac or GlcAβ3LacNAc are effectively synthesised by transglycosylation using a specific glucuronidase such as glucuronidase from bovine liver. It was realized that the enzyme can site-specifically transfer from β1-3 linkage to Galβ4GlcNAc and Galβ4Glc with unexpectedly high yields for a transglycosylation reaction. In general such selectivity and yields close 30 % or more are not obtained in transglycosylation reactions.

Another embodiment of the invention is described in Formula 9. The formula describes preferrable derivatives and analogues of the structures according to the formula 1. The formula shows also preferable modifications for producing structural analogues of the sequences.

Formula 9:



A-saccharide B-saccharide

Table 1

Binding of *Helicobacter pylori* to glycosphingolipids separated on thin-layer chromatograms.

No.	Trivial name	Glycosphingolipid structure[a]	*H. pylori* binding[b]	Source[c]	References
1	Lactotri	GlcNAcβ3Galβ4Glcβ1Cer	-	RT	(Miller-Prodraza *et al.*, 2001)
2	GgO3	GalNAcβ4Galβ4Glcβ1Cer	(+)	GPE	(Yamakawa, 1966)
3	GgO3 (de-*N*-acylated)	$GalNH_2$β4Galβ4Glcβ1Cer	-	GPE[e]	(Ångström *et al.*, 1998)
4	Le^y-6	Fucα2Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	-	DSI	(McKibbin *et al.*, 1982)
5	Neolactotetra	Galβ4GlcNAcβ3Galβ4Glcβ1Cer	(+)	HE[f]	
6	Neolactotetra (de-*N*-acylated)	Galβ4$GlcNH_2$β3Galβ4Glcβ1Cer	-	HE[e]	
7	GgO4	Galβ3GalNAcβ4Galβ4Glcβ1Cer	+	HB[g]	
8	GgO4 (de-*N*-acylated)	Galβ3$GalNH_2$β4Galβ4Glcβ1Cer	-	HB[e]	(Ångström *et al.*, 1998)
9	Le^x-5	Galβ4(Fucα3)GlcNAcβ3Galβ4Glcβ1Cer	-	DSI	(Teneberg *et al.*, 1996)
10		GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	RT[d]	(Miller-Podraza *et al.*, 2001)
11	x_2	GalNAcβ3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	HE	(Teneberg *et al.*, 1996; Thorn *et al.*, 1992)
12		GalNAcα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	HE[h]	
13	B5	Galα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	+	RE	(Eto *et al.*, 1968)
14	B5 (de-*N*-acylated)	Galα3Galβ4$GlcNH_2$β3Galβ4Glcβ1Cer	-	RE[e]	
15	P_1	Galα4Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	HE	(Naiki *et al.*, 1975)
16	H5-1	Fucα2Galβ3GlcNAcβ3Galβ4Glcβ1Cer	-	HM	(Karlsson and Larson, 1981a)
17	Le^b-6	Fucα2Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer	-	HM	(Karlsson and Larson, 1981b)
18	H5-2	Fucα2Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	HE	(Koscielak *et al.*, 1973)
19	NeuAcα3-SPG	NeuAcα3Galβ4GlcNAcβ3Galβ4Glcβ1Cer	-	HE	(Ledeen and Yu, 1978)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 03/00039

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07H 15/04, C07H 3/06, C07H 1/00, A61K 31/702, A61K 31/7028, A61K 31/715, A61K 31/726, A61P 1/04 1/16,1/18,9/00,17/00,31/04,35/00,37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07H, A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CHEM.ABS.DATA, EPO-INTERNAL, WPI DATA, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 0143751 A1 (A+ SCIENCE INVEST AB), 21 June 2001 (21.06.01) --	1-55,65
X	Glycobiology, Volume 8, No. 4, 1998, Jonas Ångström et al, "The lactosylceramide binding specificity of Helicobacter pylori", pages 297-308 --	1-55,65
X	Infection and Immunity, Volume 61, No. 6, 1993, B.D. Gold et al, "Helicobacter mustelae and Helicobacter pylori Bind to Common Lipid Receptors In Vitro", pages 2632-2638 --	1-55,65

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 June 2003	13 -06- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer PER RENSTRÖM/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI 2003/000039

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	4,10,12,61-64; 6-7,9,15-17,19,31-32, 34-35,37-43,48-56 partly	YES
	Claims	1-3,5,8,11,13-14,18,20-26,36,65	NO
Inventive step (IS)	Claims	4,10,12,61-64; 6-7,9 partly	YES
	Claims	1-3,5,8,11,13-26,31-32,34-43,48-56,65	NO
Industrial applicability (IA)	Claims	1-26,31-32,34-43,48-56,61-65	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Relevant documents (cited in the International Search Report):

D1: WO 0143751 A1(A+ SCIENCE INVEST AB), 21 June 2001 (21.06.2001)

D2: Glycobiology, Volume 8, No. 4, 1998, Jonas Ångström et al, "The lactosylceramide binding specificity of Helicobacter pylori", pages 297-308

D3: STN International, File CAPLUS, CAPLUS accession no. 2001:118779, document no. 134:292940, Fujita, M. et al, "Ancorinosides B-D, inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP), from the marine sponge Penares sollasi Thiele", & Tetrahedron, (2001), 57(7), 1229-1234

D4: STN International, File CAPLUS, CAPLUS accession no. 1998: 534525, document no. 129:258636, Miura, Yoshiaki et al, "alpha-N-Acetylgalactosamine-capping of chondroitin sulfate core region oligosaccharides primed on xylosides", & Glycobiology (1998), 8(8), 813-819

D5: STN International, File CAPLUS, CAPLUS accession no. 1994:455685, document no. 121:55685, Yamamoto, Kazuo et al, "Interaction of Immobilized Recombinant Mouse C-Type Macrophage Lectin with Glycopeptides and Oligosaccharides", & Biochemistry (1994), 33(26), 8159-66

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: V.

D6: Anger H. et al, "Amidated pectins - characterization and enzymic degradation", Food Hydrocolloids (1988), 2(5), 371-9

D7: Kratchanov, C. et al, "Reaction of apple pectin with ammonia", International Journal of Food Science and Technology (1989), 24(3) 261-7

D8: STN International, File CAPLUS, CAPLUS accession no. 1989:171879, document no. 110:171879, Anger H. et al, "Amidated pectins - characterization and enzymic degradation", & Food Hydrocolloids (1988), 2(5), 371-9

D9: WO 02056893 A1 (CARBION OY), 25 July 2002 (25.07.02)

D10: STN International, File CAPLUS, CAPLUS accession no. 2002:390032, document no. 138:1587, Chandrasekaran, E. et al, "Biosynthesis of the carbohydrate antigenic determinants, Globo E, blood group H, and Lewis b: a role for prostate cancer cell alpha1,2-L-fucosyltransferase"& Glycobiology (2002), 12(3), 153-162

D11: WO 9827988 A1 (NUTRAMAX LABORATORIES), 2 July 1998 (02.07.98)

D12: EP 0354595 A1 (UNILEVER PLC), 14 February 1990 (14.02.90)

D13: US 3405120 A (TAKEHIKO KAWANO ET AL), 8 October 1968 (08.10.68)

D14: STN International, File CAPLUS, CAPLUS accession no. 1990:512024, document no. 113:112024, Pepop. Rep. China, "Method of production of D-aminogalactose hydrochloride for clinical anlysis", & CN,A,1036386,19891018

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Previous supplemental box.

See Box III for information about which parts of the claims have not been searched or examined. See also Box VIII.

The present application is directed to Helicobacter pylori-binding substances comprising an oligosaccharide sequence of the general formula $Gal(NAc)_{r2}\beta4Glc(A)_{q2}(NAc)_{r3}$, wherein GlcA is a glucuronic acid derivative and q2, r2 and r3 are each independently 0 or 1.

D1 (abstract; page 7, lines 3-10; Table II, pages 52-53; claims) describes Helicobacter pylori-binding di- and oligosaccharide substances with the same uses as in the present application (treatment of conditions due to the presence of Helicobacter pylori (Hp) such as gastritis, ulcers, gastric adenocarcinoma, non-Hodgkin lymphoma of human stomach, liver disease, heart disease and sudden infant death syndrome; identification of bacterial adhesions; production of a Hp vaccine; diagnosis of Hp infections; typing of Hp; identifications of Hp binding substances; inhibition of Hp binding). The compounds in D1 contain the disaccharide sequence Galβ4Glc which the present application is based upon, though the focus in D1 lies on the sequences Galβ3GlcNAc and Galβ3GalNAc as binding epitopes. Specific examples are shown in Table II on pages 52-53, e.g. Galβ3GlcNAcβ3Galβ4Glcβ1Cer.

D2 describes Galβ4Glcβ1Cer (lactosylceramide), Galα3Galβ4Glcβ1Cer and Galβ3GalNAcβ4Galβ4Glcβ1Cer with binding specificity for Hp. The sequence Galβ4Glc, or at least Galβ4Glcβ1Cer, is thus known as a Hp binding epitope from D2.

D1 and D2 represent the closest prior art. The present invention can be regarded as solving the general problem of finding alternative Hp-binding substances.

Claims 1-2 and 13 cover compounds which differ from the examples in D1 (Table II, pages 52-53) and from the compounds in D2 only in that, for example, GalNac is substituted for Gal.

Claims 14-26, 31-32, 34-36, 39-40, 51-52 and 54-56, all dependent on claim 1 and subsequent claims, are found to contain only matters which are either routine practices or relate to the medical and nutritional use of Hp-binding compounds as known from the prior art of D1.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Previous supplemental box.

Claims 37 and 50 cover compounds which differ from the examples in D1 and from the compounds in D2 only in that GlcNAc is substituted for the Glc β1-bound to Cer.

Claim 38 cover compounds which differ from the examples in D1 only in that Glc is substituted for the GlcNAc β3-bound to Galβ4Glcβ1Cer and from the compounds in D2 only in that Glc is substituted for Gal (e.g. in Galα3Galβ4Glcβ1Cer).

Claim 49 cover compounds which differ from the examples in D1 only in that another monosaccharide GalNAc, GlcNAc, Gal or Glc is inserted between GlcNAcβ3Galβ4Glcβ1 and Cer.

The modifications mentioned above are considered to be minor manipulations of the known binding epitopes and to be obvious alternatives for the person skilled in the art, looking for solutions to the above mentioned problem. The person skilled in the art would expect retention of biological activity for these modifications. For example, that the difference between Galα3Galβ4Glcβ1Cer and Glcα3Galβ4Glcβ1Cer probably is insignificant with regard to binding activity can be justified by comparing with D1 (page 7, lines 3-10), in which Galβ3GlcNAc and Galβ3GalNAc are regarded as substantially interchangeable due to their structural similarity. For these reasons, and considering the broadness of the claims, the invention according to claims 1-2, 13-26, 31-32, 34-40, 49-56 is considered to lack an inventive step with regard to D1 or D2.

D3 describes a structure containing Galβ4GlcA. The invention according to claims 1, 13, 18, 20-22, 25-26, 36 and 65 lacks novelty with regard to D3. This can be avoided, for example in claim 65 if the phrase "and A indicates a glucuronamide" is inserted after the phrase "q2 is 1 and r2 is 0". Note that the claims mentioning Hp are phrased as first medical indications and thereby are to be interpreted as directed to the compounds for medical use in general, with no special regard to their Hp-binding activity.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Previous supplemental box.

D4 describes GalNAcα4GlcAβ3GalNAcβ4GlcAβ3Galβ3Galβ4Xyl (the relevant part of the structure underlined). The invention according to claims 1, 3, 5, 8, 11, 13-14, 21-22, 25-26 and 36 lacks novelty with regard to D4. Se above concerning the first medical indication.

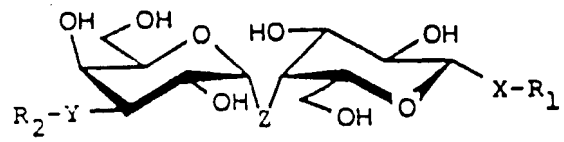
D5 describes a structure containing two terminal GalNAcβ4GlcNAc-units β2-bound to Man. The invention according to claims 1, 14, 21-22, 25-26 and 36 lacks novelty with regard to D5. Se above concerning the first medical indication.

D6-D14 only describe the general state of the art and are of no particular relevance.

The inventions defined in claims 4, 6-7, 9-10, 12 and 61-64 are not disclosed by any of the above documents, and are not obvious to a person skilled in the art. Accordingly, the inventions defined in claims 4, 6-7, 9-10, 12 and 61-64 are novel and are considered to involve an inventive step.

Claims

1. Compounds of the general formula I



I

wherein

R_1 is C_{1-24} alkyl; C_{2-24} alkenyl; C_{2-24} alkynyl; tri(C_{1-4} alkyl)silylethyl; aryl optionally substituted with hydroxy, amino, C_{1-4} alkyl, C_{1-4} alkoxy, nitro, halogen, or phenoxy; mono- or dihalogen-C_{1-4}alkyl; phenyl-C_{1-4}alkyl;

a group of the formula II or IIa

R_3-$(CH_2)_n$-$S(O)_m$-CH_2CH_2- II

$(R_3$-$(CH_2)_n$-$S(O)_m$-$CH_2)_2CH$-CH_2- IIa

wherein R_3 is H, carboxy, carboxy-C_{1-4} alkyl, hydroxy, amino, or a carrier, n is an integer from 1 to 24, and m is 0 or 2;

a group of the formula III or IIIa

Phe-$S(O)_m$-CH_2CH_2- III

$(Phe$-$S(O)_m$-$CH_2)_2CH$-CH_2- IIIa

wherein m is as defined above, and each Phe is a phenyl group optionally monosubstituted with hydroxy, amino, C_{1-4} alkyl, C_{1-4} alkoxy, nitro, halogen, or phenoxy

a group of the formula IV

$R_4CH_2CH(CH_2R_5)CH_2$- IV

wherein R_4 and R_5 independently are halogen;

a group Q-$(CH_2)_n$- where Q is a carrier, and n is as defined above; and

R_2 is a mono- or disaccharide moiety connected via a glycosidic bond; C_{1-18} alkyl; C_{2-18} alkenyl; C_{2-18} alkynyl; C_{1-18} alkyloxymethyl; C_{1-18} alkanoyl; α-hydroxy-C_{1-18} alkanoyl; naphthyl-, heterocyclyl- or phenyl-C_{1-8} alkoxy where the naphthyl, heterocyclyl or phenyl group may be substituted with hydroxy, amino, C_{1-4} alkyl, C_{1-4}alkoxy, nitro, halogen, or phenoxy; tri(C_{1-4}-alkyl)-silylethyl; tri(C_{1-4}-alkyl)silyl; tri(C_{1-4}-alkyl)silylethoxymethyl; halogen; ω-hydroxy-C_{1-4}alkyl; tetrahydropyranyl; benzyloxymethyl; C_{3-8} cycloalkyl; a monoterpenyl moiety; benzoyl optionally monosubstituted with hydroxy, amino, C_{1-4} alkyl, C_{1-4} alkoxy, nitro, halogen, or phenoxy; the acyl residue of a naturally occurring amino acid; or a group of the formula V

R_6-$C(O)$-$N(R_7)$-$CH(R_8)$-CH_2- V

wherein R_6 is C_{1-4} alkyl; or phenyl optionally monosubstituted with hydroxy, amino, C_{1-4} alkyl, C_{1-4} alkoxy, nitro, halogen, or phenoxy,

R_7 is H or C_{1-4} alkyl, and

R_8 is H, C_{1-4} alkyl, or hydroxy-C_{1-4} alkyl,

Z is -O-, -S-, -SO_2-, or -CH_2-,

X is -O-, -S-, -SO_2-, -CH_2-, or -NR_3-, wherein R_3 is H or is one of the meanings for R_2 above, R_3 and R_1 optionally being connected to form a ring, and

Y is -O- or -NR_3- where R_3 is H or is one of the meanings for R_2 above, R_3 and R_2 optionally being connected to form a ring.

Publication Number	Application Number	Doc Kind	Pages	Country
02056893	0200043	A1	1	WO

Publication Date	Application Date	Emperor Code
Jul 25, 2002	Jan 18,2002	

OK | Print Detail

Priority Number	Inventor
20010118	NATUNEN JARI

Int'l Classification	Patent Applicant
A61K31/702	CARBION OY

Title Of Invention

NOVEL RECEPTORS FOR DOLLAR I(HELICOBACTER PYLORI) AND USE THEREOF
NOUVEAUX R¿CEPTEURS POUR DOLLAR I(HELICOBACTER PYLORI) ET LEUR UTILISATION

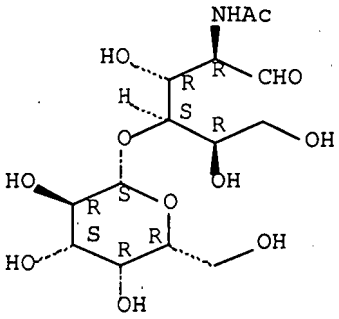
Time Left: Account Tuesday, Sep 18 2007, 4:02:03 PM

L85 ANSWER 2 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 8
ACCESSION NUMBER: 1998:222395 HCAPLUS Full-text
DOCUMENT NUMBER: 128:321033
TITLE: Inhibition of Helicobacter pylori and Helicobacter mustelae binding to lipid receptors by bovine colostrum
AUTHOR(S): Bitzan, Martin M.; Gold, Benjamin D.; Philpott, Dana J.; Huesca, Mario; Sherman, Philip M.; Karch, Helge; Lissner, Reinhard; Lingwood, Clifford A.; Karmali, Mohamed A.
CORPORATE SOURCE: Division of Microbiology, University of Toronto, Ontario, Can.
SOURCE: Journal of Infectious Diseases (1998), 177(4), 955-961
CODEN: JIDIAQ; ISSN: 0022-1899
PUBLISHER: University of Chicago Press
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 22 Apr 1998
AB Helicobacter pylori, the etiol. agent of chronic-active gastritis and duodenal ulcers in humans, and Helicobacter mustelae, a gastric pathogen in ferrets, bind to phosphatidylethanolamine (PE), a constituent of host gastric mucosal cells, and to gangliotetraosylceramide (Gg4) and gangliotriaosylceramide (Gg3). The effect of a bovine colostrum concentrate (BCC) on the interaction of H. pylori and H. mustelae to their lipid receptors was examined BCC blocked attachment of both species to Gg4, Gg3, and PE. Partial inhibition of binding was observed with native bovine and human colostra. BCC lacked detectable antibodies (by immunoblotting) to H. pylori surface proteins (adhesins). However, colostral lipid exts. contained PE and lyso-PE that bound H. pylori in vitro. These results indicate that colostrum can block the binding of Helicobacter species to select lipids and that binding inhibition is conferred, in part, by colostral PE or PE derivs. Colostral lipids may modulate the interaction of H. pylori and other adhesin-expressing pathogens with their target tissues.
CC 18-7 (Animal Nutrition)
Section cross-reference(s): 1, 15
IT Antimicrobial agents
Colostrum
Helicobacter mustelae
Helicobacter pylori
(inhibition of Helicobacter pylori and Helicobacter mustelae binding to lipid receptors by colostrum from humans and cows)
IT 35960-33-9, Gangliotriaosylceramide 71012-19-6, Gangliotetraosylceramide
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(inhibition of Helicobacter pylori and Helicobacter mustelae binding to lipid receptors by colostrum from humans and cows)
IT 35960-33-9, Gangliotriaosylceramide 71012-19-6, Gangliotetraosylceramide
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(inhibition of Helicobacter pylori and Helicobacter mustelae binding to lipid receptors by colostrum from humans and cows)
RN 35960-33-9 HCAPLUS
CN Ceramide, 1-O-[O-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]- (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
RN 71012-19-6 HCAPLUS
CN Ceramide, 1-O-[O-β-D-galactopyranosyl-(1→3)-O-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]- (CA INDEX NAME)

L85 ANSWER 3 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 1998:119175 HCAPLUS Full-text
DOCUMENT NUMBER: 128:241583
TITLE: Glucosylated N-acetyllactosamine O-antigen chain in the lipopolysaccharide from Helicobacter pylori strain UA861
AUTHOR(S): Monteiro, Mario A.; Rasko, David; Taylor, Diane E.; Perry, Malcolm B.
CORPORATE SOURCE: Institute for Biological Sciences, National Research Council, Ottawa, ON, K1A OR6, Can.
SOURCE: Glycobiology (1998), 8(1), 107-112
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 27 Feb 1998
AB The O-antigen chain from the lipopolysaccharide of Helicobacter pylori strain UA861 was determined to be composed of an elongated type 2 N-acetyllactosamine backbone, -[→3)-β-D-Gal-(1→4)- β-D-GlcNAc-(1-]n→, with approx. half of the GlcNAc units carrying a terminal α-D-Glc residue at the 0-6 position. The O-chain of H. pylori UA861 was terminated by a N-acetyllactosamine [β-D-Gal-(1→4)-β-D-GlcNAc] (LacNAc) epitope and did not express terminal Lewis X or Lewis Y blood-group determinants as previously found in other H.pylori strains. The absence of terminal Lewis X and Lewis Y blood-group epitopes and the replacement of Fuc by Glc as a side chain in the O-chain of H .pylori UA861 represents yet another type of lipopolysaccharide structure from H. pylori species. These structural differences in H. pylori lipopolysaccharide mols. carry implications with regard to possible different pathogenic events between strains and resp. hosts.
CC 10-1 (Microbial, Algal, and Fungal Biochemistry)
Section cross-reference(s): 15
IT Epitopes
Helicobacter pylori
(glucosylated acetyllactosamine O-antigen chain in lipopolysaccharide from Helicobacter pylori)
IT 32181-59-2, N-Acetyllactosamine
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(glucosylated acetyllactosamine O-antigen chain in lipopolysaccharide from Helicobacter pylori)
IT 32181-59-2, N-Acetyllactosamine
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(glucosylated acetyllactosamine O-antigen chain in lipopolysaccharide from Helicobacter pylori)
RN 32181-59-2 HCAPLUS
CN D-Glucose, 2-(acetylamino)-2-deoxy-4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry.


NHAc
HO
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R
CHO
H
S
R
O
OH
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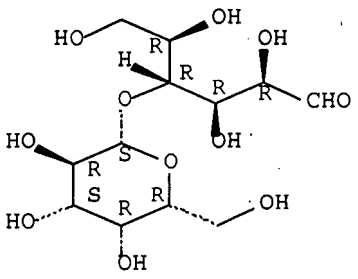
OH
HO
OH

L85 ANSWER 11 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:455031 HCAPLUS Full-text
DOCUMENT NUMBER: 139:30764
TITLE: Methods and compositions for stimulating secretions from Paneth cells
INVENTOR(S): Zasloff, Michael A.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 5 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003109582	A1	20030612	US 2002-313261	20021206 <--
PRIORITY APPLN. INFO.:			US 2001-337824P P	20011210 <--

ED Entered STN: 13 Jun 2003
AB Methods and compns. are disclosed for stimulating Paneth cells to release natural antimicrobial agents including peptides, to reduce or eliminate pathogenic organisms in the GI tract of mammalian bodies, including humans, utilizing an active isoleucine compound as a secretagogue.
IC ICM A61K031-198
INCL 514561000
CC 1-5 (Pharmacology)
Section cross-reference(s): 2, 18, 63
IT Helicobacter pylori
(associated ulcers; methods and compns. for stimulating secretions from Paneth cells)
IT 63-42-3, Lactose
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(intolerance; methods and compns. for stimulating secretions from Paneth cells)
IT 63-42-3, Lactose
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(intolerance; methods and compns. for stimulating secretions from Paneth cells)
RN 63-42-3 HCAPLUS
CN D-Glucose, 4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



L85 ANSWER 12 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:77718 HCAPLUS Full-text
DOCUMENT NUMBER: 138:126995
TITLE: Coated granules of allylamine- or benzylamine-antimycotics, process for preparation thereof and orodispersible tablets containing said coated granules
INVENTOR(S): Cousin, Gerard; Bruna, Etienne; Gendrot, Edouard
PATENT ASSIGNEE(S): Ethypharm, Fr.
SOURCE: Eur. Pat. Appl., 16 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1279402	A1	20030129	EP 2001-402027	20010726 <--
EP 1279402	B1	20061129		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
AT 346590	T	20061215	AT 2001-402027	20010726 <--
WO 2003009830	A1	20030206	WO 2002-EP8103	20020719 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002328930	A1	20030217	AU 2002-328930	20020719 <--
JP 2005503366	T	20050203	JP 2003-515223	20020719 <--
US 2004220276	A1	20041104	US 2004-484588	20040123 <--
PRIORITY APPLN. INFO.:			EP 2001-402027 A	20010726 <--
			WO 2002-EP8103 W	20020719 <--

ED Entered STN: 31 Jan 2003

AB Granules of allylamine- or benzylamine-ant-mycotic or one of its pharmaceutically acceptable salts, are characterized in that the granules are coated and that they contain microcrystals of an allylamine- or benzylamine-antimycotic or one of its pharmaceutically acceptable salts, at least one binder selected from the group consisting of cellulosic polymers, such as Et cellulose, hydroxypropyl cellulose and hydroxypropyl Me cellulose, acrylic polymers, povidones, polyvinylalcs. and mixts. thereof, optionally a diluent agent selected from the group comprising cellulosic derivs., starches, lactose, polyols such as mannitol and/or an antistatic agent selected from the group comprising micronized or non-micronized talc and/or a permeabilizing agent selected from the group comprising colloidal silica and precipitated silica. Also disclosed is a process for obtaining such granules and orodispersible tablets comprising them. Terbinafine·HCl was granulated in a fluidized bed with ethanol in which PVP was dissolved. The granules were coated by spraying thereon HPMC in water and Aquacoat ECD30/triethyl citrate to the amount of 30 % with respect to the weight of the granules of terbinafine·HCl. More than 80 % of terbinafine was dissolved after 10 min and taste-masking was efficient.

IC ICM A61K009-20
ICS A61K009-16
CC 63-6 (Pharmaceuticals)

IT Candida albicans
Helicobacter pylori
(infection with, treatment of; manufacture of coated antimycotic granules and orodispersible tablets containing the same)

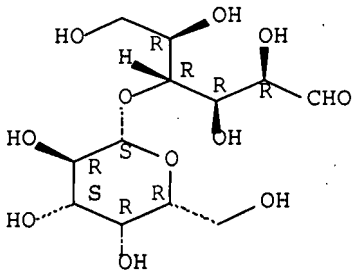
IT 63-42-3, Lactose 69-65-8, D-Mannitol 7585-39-9, β-Cyclodextrin 7631-86-9, Silica, biological studies 9002-89-5, Polyvinyl alcohol 9003-39-8, Povidone 9004-34-6, Cellulose, biological studies 9004-57-3, Ethyl cellulose 9004-64-2, Hydroxypropyl cellulose 9004-65-3, Hydroxypropyl methyl cellulose 9005-25-8, Starch, biological studies 9050-36-6, Maltodextrin 14807-96-6, Talc, biological studies 24938-16-7, Eudragit E100 25086-15-1, Eudragit L100 25212-88-8, Eudragit L30D-55 65472-88-0, Naftifine 74811-65-7, Sodium Croscarmellose 78628-80-5, Terbinafine hydrochloride 91161-71-6, Terbinafine 101828-21-1, Butenafine
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(manufacture of coated antimycotic granules and orodispersible tablets containing the same)

IT 63-42-3, Lactose
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(manufacture of coated antimycotic granules and orodispersible tablets containing the same)

RN 63-42-3 HCAPLUS

CN D-Glucose, 4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



L85 ANSWER 16 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:391563 HCAPLUS Full-text
DOCUMENT NUMBER: 136:391021
TITLE: Colostrum-based pharmaceutical compositions
INVENTOR(S): Williams, Charles Edward; Hobman, Peter Graeme; Yarrow, Simon Stephen
PATENT ASSIGNEE(S): Fonterra Co-Operative Group Limited, N. Z.
SOURCE: PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002040051	A1	20020523	WO 2001-NZ256	20011115 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 200224240	A	20020527	AU 2002-24240	20011115 <--
EP 1341554	A1	20030910	EP 2001-996393	20011115 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2004517067	T	20040610	JP 2002-542423	20011115 <--
HU 200400589	A2	20040628	HU 2004-589	20011115 <--
HU 200400589	A3	20050628		
US 2004047856	A1	20040311	US 2003-416831	20031008 <--
US 2005220894	A1	20051006	US 2005-136575	20050525 <--
PRIORITY APPLN. INFO.:			NZ 2000-508234 A	20001115 <--
			WO 2001-NZ256 W	20011115 <--
			US 2003-416831 A3	20031008 <--

ED Entered STN: 24 May 2002

AB A composition including colostrum or a colostrum-derived product and hyperimmune milk (HIM) or a hyperimmune milk-derived product, in amts. sufficient to provide a combined spectrum of pathogen-binding activity against a broad-spectrum of pathogenic organisms is described. For example, a test composition was prepared including 70% colostrum milk protein powder, 24% hyperimmune milk powder, 4% ganglioside-containing component, whey powder, lactose and 1.5% milk calcium. The test composition of the invention includes a combination of ingredients each of which has particular antimicrobial binding and/or anti-inflammatory activity which may combine to produce particular and unexpected clin. benefits in a broad range of diseases, including infection-associated diseases, and particularly gastrointestinal, inflammatory and bone related disorders. Such benefits are an unexpected result of the combination used.

IC ICM A61K045-06
ICS A61K039-395; A61P029-00; A61P031-04; A61P001-00
CC 63-6 (Pharmaceuticals)
Section cross-reference(s): 1, 15, 17
IT Anti-inflammatory agents
Antiarthritics
Antimicrobial agents
Beverages

Candida albicans
Clostridium difficile
Escherichia coli
Helicobacter pylori
Klebsiella pneumoniae
Salmonella typhimurium
(colostrum-based compns. with anti-inflammatory and antimicrobial activities)

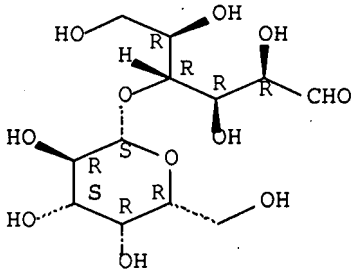
IT 63-42-3, Lactose 7440-70-2, Calcium, biological studies 54827-14-4, Ganglioside GM3 62010-37-1, Ganglioside GD3 67763-96-6, IGF-1
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(colostrum-based compns. with anti-inflammatory and antimicrobial activities)

IT 63-42-3, Lactose
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(colostrum-based compns. with anti-inflammatory and antimicrobial activities)

RN 63-42-3 HCAPLUS

CN D-Glucose, 4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



L85 ANSWER 17 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:946833 HCAPLUS Full-text
DOCUMENT NUMBER: 138:16581
TITLE: Use of bacterial phage-associated lysing enzymes for treating various illnesses
INVENTOR(S): Loomis, Lawrence; Fischetti, Vincent
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 25 pp., Cont.-in-part of U.S. Pat. Appl. 2001 13,931.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002187136	A1	20021212	US 2001-844435	20010430 <--
US 6752988	B1	20040622	US 2000-560650	20000428 <--
US 2001013931	A1	20010816	US 2001-752732	20010103 <--
US 6583872	B2	20030624		
US 2005136088	A1	20050623	US 2004-855978	20040528 <--
PRIORITY APPLN. INFO.:			US 2000-560650 A2	20000428 <--
			US 2001-752732 A2	20010103 <--
			JP 2000-35724 A	20000214 <--
			US 2001-844435 B1	20010430 <--

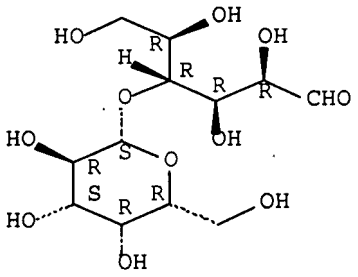
ED Entered STN: 13 Dec 2002
AB A composition and method for treating bacterial infections is disclosed which comprises the treatment of an individual with an effective amount of at least one lytic enzyme produced by bacteria infected with a bacteriophage specific for said bacteria wherein at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, holin enzymes, and combinations thereof. A carrier may be used for delivering the lytic enzyme. This method, and composition can be used for the treatment of upper respiratory infections, skin infections, wounds, and burns, vaginal infections, eye infections, intestinal disorders and dental problems.
IC ICM A61K038-43
INCL 424094100
CC 63-3 (Pharmaceuticals)
Section cross-reference(s): 62
IT Campylobacter
Escherichia coli
Haemophilus influenzae
Helicobacter pylori
Listeria
Mycobacterium tuberculosis
Pseudomonas
Salmonella
Streptococcus mutans
Streptococcus pneumoniae
(infection by; use of bacterial phage-associated lysing enzymes for treating various illnesses)
IT 50-70-4, Sorbitol, biological studies 50-99-7, Dextrose, biological studies 63-42-3, Lactose 67-68-5, Dmso, biological studies 69-65-8, Mannitol 7647-14-5, Sodium chloride, biological studies
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(use of bacterial phage-associated lysing enzymes for treating various illnesses)
IT 63-42-3, Lactose

RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(use of bacterial phage-associated lysing enzymes for treating various illnesses)

RN 63-42-3 HCAPLUS

CN D-Glucose, 4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



L85 ANSWER 19 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2002:894761 HCAPLUS Full-text
DOCUMENT NUMBER: 138:1667
TITLE: Preparation of Helicobacter pylori glucose/galactose transferase for glycoconjugate biosynthesis
INVENTOR(S): Endo, Tetsuo; Koizumi, Satoshi
PATENT ASSIGNEE(S): Kyowa Hakko Kogyo Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 15 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

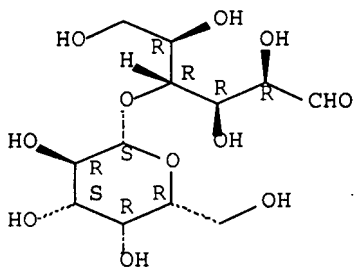
PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002335969	A	20021126	JP 2001-147239	20010517 <--
PRIORITY APPLN. INFO.:			JP 2001-147239	20010517 <--

ED Entered STN: 26 Nov 2002

AB This invention provides a process of preparation of Helicobacter pylori glucose/galactose transferase. The DNA and protein sequences of Helicobacter pylori glucose/galactose transferase were disclosed. The enzyme can be used for biosynthesis of glycoconjugate.

IC ICM C12N015-09
ICS C12N001-21; C12N005-10; C12N009-10; C12P019-18
CC 7-2 (Enzymes)
Section cross-reference(s): 3, 10
IT Helicobacter pylori
(glucose/galactose transferase cloned from; preparation of Helicobacter pylori glucose/galactose transferase for glycoconjugate biosynthesis)
IT 63-42-3, Lactose 13007-32-4, Lacto-N-neotetraose 14116-68-8, Lacto-N-tetraose 32181-59-2, N-Acetyllactosamine 66580-68-5, Globotriose
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(receptor of glucose and galactose; preparation of Helicobacter pylori glucose/galactose transferase for glycoconjugate biosynthesis)
IT 63-42-3, Lactose 13007-32-4, Lacto-N-neotetraose 14116-68-8, Lacto-N-tetraose 32181-59-2, N-Acetyllactosamine 66580-68-5, Globotriose
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(receptor of glucose and galactose; preparation of Helicobacter pylori glucose/galactose transferase for glycoconjugate biosynthesis)
RN 63-42-3 HCAPLUS
CN D-Glucose, 4-O-β-D-galactopyranosyl- (CA INDEX NAME)

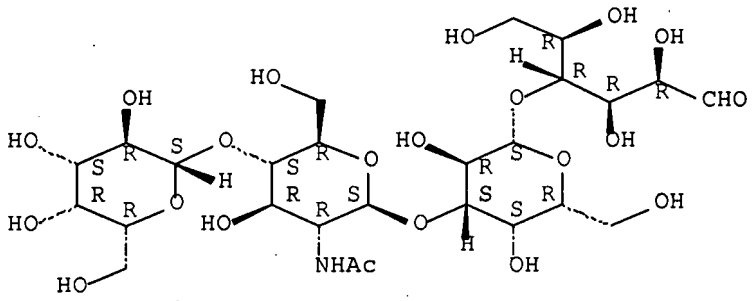
Absolute stereochemistry. Rotation (+).



RN 13007-32-4 HCAPLUS

CN D-Glucose, O-β-D-galactopyranosyl-(1→4)-O-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)- (CA INDEX NAME)

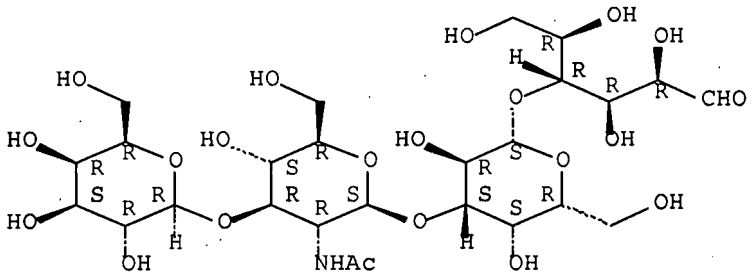
Absolute stereochemistry.



RN 14116-68-8 HCAPLUS

CN D-Glucose, O-β-D-galactopyranosyl-(1→3)-O-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)- (CA INDEX NAME)

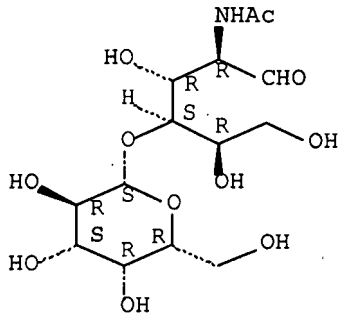
Absolute stereochemistry.



RN 32181-59-2 HCAPLUS

CN D-Glucose, 2-(acetylamino)-2-deoxy-4-O-β-D-galactopyranosyl- (CA INDEX NAME)

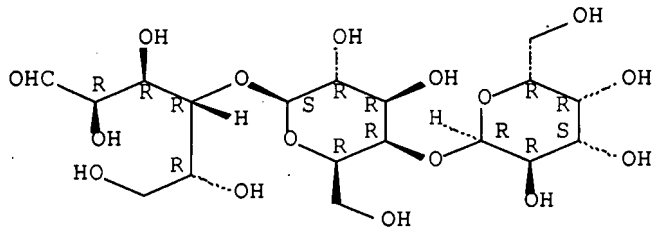
Absolute stereochemistry.



RN 66580-68-5 HCAPLUS

CN D-Glucose, O-α-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



L85 ANSWER 24 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:472908 HCAPLUS Full-text
DOCUMENT NUMBER: 135:72142
TITLE: Modified Helicobacter pylori α-1,2-fucosyltransferase gene and use in fucose-containing sugar biosynthesis
INVENTOR(S): Endo, Tetsuo; Koizumi, Satoshi; Tabata, Kazuhiko; Ozaki, Akio
PATENT ASSIGNEE(S): Kyowa Hakko Kogyo Co., Ltd., Japan
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001046400	A1	20010628	WO 2000-JP9033	20001220 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2395076	A1	20010628	CA 2000-2395076	20001220 <--
AU 2001022216	A5	20010703	AU 2001-22216	20001220 <--
EP 1243647	A1	20020925	EP 2000-985799	20001220 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: JP 1999-362243 A 19991221 <--
WO 2000-JP9033 W 20001220 <--

ED Entered STN: 29 Jun 2001

AB Recombinant DNA coding for Helicobacter pylori α1,2- fucosyltransferase (FucT) with modification in poly(C) sequence, TAA repeats, or AAAAAAG sequences, and designed with preferred codon usage, and use in biosynthesis of fucose-containing oligosaccharides, are disclosed. A fucose-containing sugar can be economically produced in a large amount by bringing a acceptor sugar into contact with a microorganism capable of producing GTP from a GTP precursor and a microorganism capable of producing GDP-fucose from a sugar and GTP in an aqueous medium. The acceptor sugar is an oligosaccharide containing galactose at the non-reducing end. The oligosaccharide moiety is either lactose, N-acetyl lactosamine, Lewis X, or Lewis a. A fucose-containing sugar such as fucosyl lactose, fucosyl N-acetyl lactosamine, Lewis Y, or Lewis b are produced. GTP precursors such as guanine, xanthine, hypoxanthine, guanosine, xanthosine, inosine, guanosine-5'-monophosphate, xanthosine-5'-monophosphate, or inosine-5'-monophosphate, can be used. Glucose, fructose, or mannose can be used for GDP-fucose production Corynebacteria such as Corynebacterium ammoniagenes can be used. Microorganism having elevated activity of glucokinase (glk gene), phosphomannomutase (manB gene), mannose-1-phosphate guaniryltransferase (manC gene), phosphoglucomutase (pgm gene), phosphofructokinase (pfk gene), GDP-mannose 4,6-dehydratase (gmd gene), or GKDM epimerase/reductase (wcaG gene), can be used. Helicobacter pylori lipopolysaccharides (LPS) contain complex carbohydrates known as Lewis antigens which may contribute to the pathogenesis and adaptation of the bacterium. Involved in the biosynthesis of Lewis antigens is an α1,2-

fucosyltransferase (FucT) that adds fucose to the terminal βGal unit of the O-chain of LPS. Recently, the H. pylori (Hp) α1,2-FucT-encoding gene (fucT2) was cloned and analyzed in detail. In contrast to the normal mammalian α1,2-FucT (H or Se enzyme), Hp α1,2-FucT prefers to use Lewis X [βGal1-4(αFuc1-3)βGlcNAc] rather than LacNAc [βGal1-4βGlcNAc] as a substrate, suggesting that H. pylori uses a novel pathway (via Lewis X) to synthesize Lewis Y. Hp α1,2-FucT also acts on type 1 acceptor [βGal1-3βGlcNAc] and Lewis a [βGal1-3(αFuc1-4)βGlcNAc], which provides H. pylori with the potential to synthesize H type 1 and Lewis b epitopes. The ability to transfer fucose to a monofucosylated substrate (Lewis X or Lewis a) makes Hp α1,2-FucT distinct from normal mammalian α1,2-FucT.

IC ICM C12N015-09
ICS C12N001-21; C12N009-10; C12P019-18; C12N001-21; C12R001-19

CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 7, 10

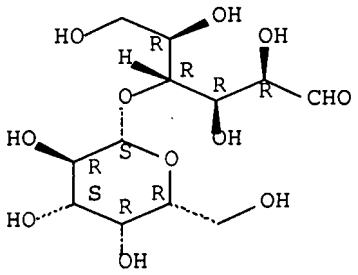
IT Codon usage
DNA sequences
Helicobacter pylori
(modified Helicobacter pylori α-1,2-fucosyltransferase gene and use in fucose-containing sugar biosynthesis)

IT 63-42-3, Lactose 32181-59-2, N-Acetyl lactosamine
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(oligosaccharide moiety of acceptor; modified Helicobacter pylori α-1,2-fucosyltransferase gene and use in fucose-containing sugar biosynthesis)

IT 63-42-3, Lactose 32181-59-2, N-Acetyl lactosamine
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(oligosaccharide moiety of acceptor; modified Helicobacter pylori α-1,2-fucosyltransferase gene and use in fucose-containing sugar biosynthesis)

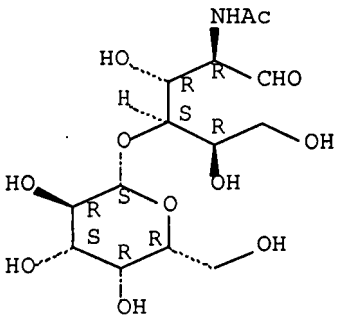
RN 63-42-3 HCAPLUS
CN D-Glucose, 4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry. Rotation (+).



RN 32181-59-2 HCAPLUS
CN D-Glucose, 2-(acetylamino)-2-deoxy-4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry.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OH
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HO
OH

L85 ANSWER 25 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:208394 HCAPLUS Full-text
DOCUMENT NUMBER: 134:247231
TITLE: Transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection
INVENTOR(S): Paton, Adrienne; Morona, Renato; Paton, James
PATENT ASSIGNEE(S): Women's and Children's Hospital, Australia; Luminis Pty Ltd.
SOURCE: PCT Int. Appl., 94 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001019960	A1	20010322	WO 2000-IB1349	20000909 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW				
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CA 2384081	A1	20010322	CA 2000-2384081	20000909 <--
EP 1214396	A1	20020619	EP 2000-958947	20000909 <--
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BR 2000013915	A	20021119	BR 2000-13915	20000909 <--
HU 200202863	A2	20021228	HU 2002-2863	20000909 <--
JP 2003514512	T	20030422	JP 2001-523732	20000909 <--
NZ 517821	A	20040130	NZ 2000-517821	20000909 <--
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MX 2002PA02536	A	20030721	MX 2002-PA2536	20020308 <--
IN 2002DN00287	A	20070302	IN 2002-DN287	20020308 <--
US 2005250196	A1	20051110	US 2004-18745	20041220 <--
PRIORITY APPLN. INFO.:			AU 1999-2757 A	19990910 <--
			US 2000-658537 A1	20000909 <--
			WO 2000-IB1349 W	20000909 <--

ED Entered STN: 22 Mar 2001
AB Transgenic microorganisms that carry mimics of the endogenous carbohydrate ligand for a bacterial toxin or virulence factor are described for use in the control of infection or intoxication. These microorganisms can be used as a means to competitively inhibit the binding of toxins or adhesins to receptors of mucosal surfaces, especially gastrointestinal surface. In particular chimeric sugar moieties have been made for lipopolysaccharides, in recombinant microorganism that present multiple copies of the oligosaccharides. The oligosaccharide moieties so presented act as receptor mimic for toxins and adhesins. A number have been synthesized and have been shown to confer protection against attack by pathogenic organisms or their products in vitro and an in vivo.
IC ICM C12N001-21
ICS A61K031-7028; A61K031-702; A61K035-74.
CC 1-5 (Pharmacology)

Section cross-reference(s): 3, 10

IT Acanthamoeba
Candida albicans
Chlamydia trachomatis
Entamoeba histolytica
Haemophilus influenzae
Haemophilus parainfluenzae
Helicobacter pylori
Pseudomonas
Streptococcus pneumoniae
(adhesin ligand mimics for control of infection by; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)

IT 131-48-6, N-Acetylneuraminic acid 499-40-1 1811-31-0, N-Acetylgalactosamine 3371-50-4 13117-26-5 24656-24-4 29923-15-7 41744-59-6 54827-14-4D, GM3, NeuNAc and NeuGc derivs. 330624-92-5
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(as inhibitor of bacterial binding to animal cells; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)

IT 37758-47-7, GM1 71012-19-6, Asialo-GM1
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(inhibition of heat labile enterotoxin binding to animal cells via; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)

IT 13007-32-4, Lacto-N-neotetraose 77356-46-8
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(manufacture of, as inhibitor of Clostridium binding to animal cells; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)

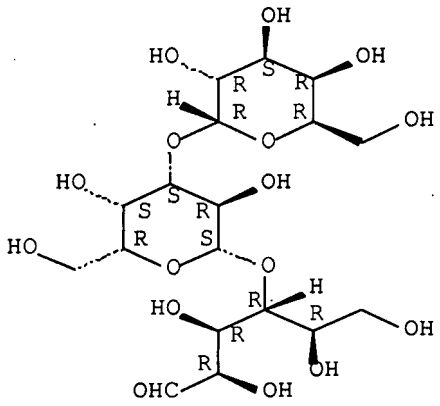
IT 32181-59-2 66580-68-5 75660-79-6, Globotetraose
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(manufacture of, as inhibitor of Shiga toxin binding to animal cells; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)

IT 41744-59-6
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(as inhibitor of bacterial binding to animal cells; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)

RN 41744-59-6 HCAPLUS

CN D-Glucose, O-α-D-galactopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)- (CA INDEX NAME)

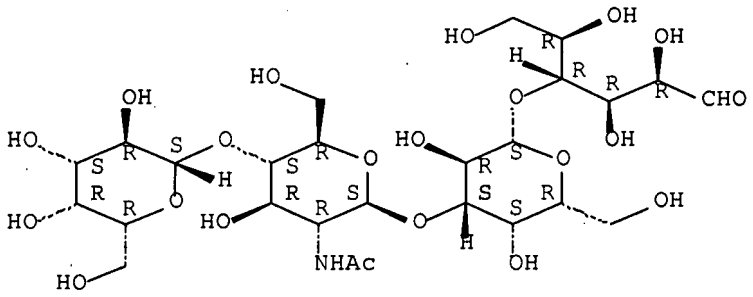
Absolute stereochemistry.



IT 71012-19-6, Asialo-GM1
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(inhibition of heat labile enterotoxin binding to animal cells via; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)
RN 71012-19-6 HCAPLUS
CN Ceramide, 1-O-[O-β-D-galactopyranosyl-(1→3)-O-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]- (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
IT 13007-32-4, Lacto-N-neotetraose 77356-46-8
RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(manufacture of, as inhibitor of Clostridium binding to animal cells; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)
RN 13007-32-4 HCAPLUS
CN D-Glucose, O-β-D-galactopyranosyl-(1→4)-O-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)- (CA INDEX NAME)

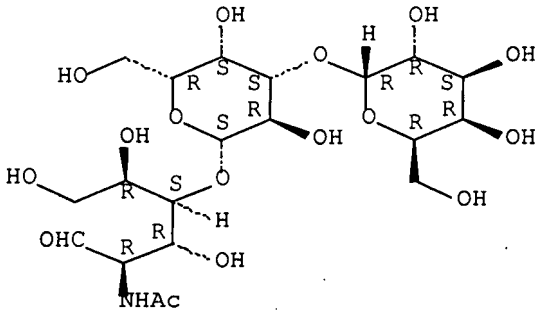
Absolute stereochemistry.



RN 77356-46-8 HCAPLUS

CN D-Glucose, O-α-D-galactopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-2-(acetylamino)-2-deoxy- (CA INDEX NAME)

Absolute stereochemistry.



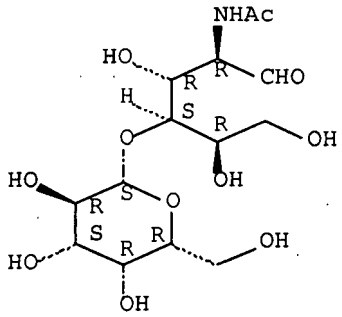
IT 32181-59-2 66580-68-5

RL: BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process); USES (Uses)
(manufacture of, as inhibitor of Shiga toxin binding to animal cells; transgenic microorganisms presenting mimics of mammalian adhesin-binding oligosaccharides on their surfaces and their use in controlling infection)

RN 32181-59-2 HCAPLUS

CN D-Glucose, 2-(acetylamino)-2-deoxy-4-O-β-D-galactopyranosyl- (CA INDEX NAME)

Absolute stereochemistry.



RN 66580-68-5 HCAPLUS

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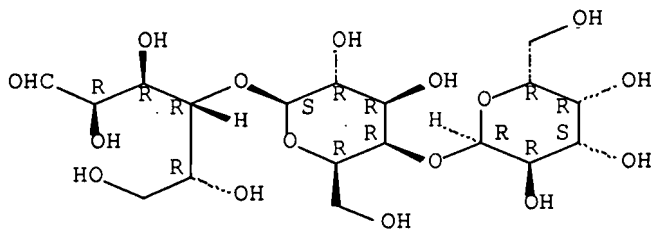
Absolute stereochemistry.



L85 ANSWER 26 OF 102 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2001:914809 HCAPLUS Full-text
DOCUMENT NUMBER: 136:181602
TITLE: In vitro binding of Helicobacter pylori to monohexosylceramides
AUTHOR(S): Abul-Milh, Maan; Barnett Foster, Debora; Lingwood, C. A.
CORPORATE SOURCE: Department of Applied Chemical and Biological Sciences, Ryerson Polytechnic University, Toronto, ON, M5B 2K3, Can.
SOURCE: Glycoconjugate Journal (2001), 18(3), 253-260
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
ED Entered STN: 19 Dec 2001
AB H. pylori is the major cause of human gastritis, duodenal ulcer and thus gastric adenocarcinoma. Many glycosphingolipid species have been postulated as receptors for H. pylori and it is likely that H. pylori attachment requires multiple, perhaps sequential receptor/ligand interactions. In this study, the binding of a number of H. pylori clin. isolates, as well as stock strains, to acid and neutral glycosphingolipids separated on thin-layer chromatograms was characterized under microaerobic conditions. All H. pylori clin. isolates, laboratory strains and type culture collection strains recognized galactosylceramide (Galβ1Cer) with ceramide containing sphingosine and hydroxylated fatty acid (type I), or nonhydroxylated fatty acid (type II), on thin-layer chromatograms and when incorporated into liposomes. The clin. isolates bound stronger to Galβ1Cer (type II) than Galβ1Cer (type I) on TLC, whereas laboratory and culture collection strains showed the opposite binding preference. A clear preference in binding to Galβ1Cer (type I) incorporated into liposome was shown by most tested strains. Clin. isolates bound well to glucosylceramide (Glcβ1Cer) with hydroxylated fatty acid, whereas weak binding to this glycolipid was detected with the laboratory and type collection strains. None of the tested strains bound Glcβ1Cer with non-hydroxylated fatty acid on the solid surface, but some strains of both clin. or type collection origins showed weak or very weak binding in the liposome assay. A clear distinction between the binding specificity of living organisms (under microaerobic conditions) as opposed to dying organisms (under normoxic conditions) illustrates the importance of cellular physiol. in this process. These studies illustrate lipid modulation of the potential receptor function of monohexosylceramides and the distinction between the receptor repertoire of H. pylori clin. isolates and cultured strains commonly used to study host-cell adhesion.
CC 14-3 (Mammalian Pathological Biochemistry)
Section cross-reference(s): 10
IT Adhesion, biological
Helicobacter pylori
Human
(Helicobacter pylori binding to glycosphingolipids in model of host-cell adhesion)
IT 4682-48-8, Lactosylceramide 35960-33-9, Gangliotriaosylceramide 71012-19-6, Gangliotetraosylceramide 85305-87-9, Glucosylceramide 85305-88-0, Galactosylceramide 89678-50-2, Ganglioside GM3
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Helicobacter pylori binding to glycosphingolipids in model of host-cell adhesion)
IT 35960-33-9, Gangliotriaosylceramide 71012-19-6,

Gangliotetraosylceramide
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Helicobacter pylori binding to glycosphingolipids
in model of host-cell adhesion)

RN 35960-33-9 HCAPLUS

CN Ceramide, 1-O-[O-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]- (CA INDEX NAME)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

RN 71012-19-6 HCAPLUS

CN Ceramide, 1-O-[O-β-D-galactopyranosyl-(1→3)-O-2-(acetylamino)-2-deoxy-β-D-galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl]- (CA INDEX NAME)

Interaction of Immobilized Recombinant Mouse C-Type Macrophage Lectin with Glycopeptides and Oligosaccharides†

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Division of Chemical Toxicology and Immunochemistry, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113, Japan, and Pharmacia Biotech (Japan) Inc., Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

Received February 7, 1994; Revised Manuscript Received May 3, 1994•

ABSTRACT: Inflammatory and tumoricidal macrophages express galactose- and *N*-acetylgalactosamine-specific Ca^{2+}-dependent lectins on their surfaces. This lectin is a family member of membrane-bound C-type animal lectins and consists of 304 amino acid residues (molecular weight 34 595). In the present study, expression vectors containing a nucleotide sequence corresponding to the carbohydrate-binding domain of mouse macrophage lectin cDNA have been prepared. The carbohydrate-binding specificity of the recombinant macrophage lectin expressed in *Escherichia coli* was investigated by comparing elution profiles of various glycopeptides having defined carbohydrate structures on immobilized lectins. When elution profiles of high mannose-type and complex-type Asn-linked carbohydrate chains were compared, the degree of retardation from immobilized macrophage lectin column was in the order tetraantennary complex-type with terminal galactosyl residues > triantennary complex-type with terminal galactosyl residues > biantennary complex-type with terminal galactosyl residues > high mannose-type glycopeptides. N-Terminal octapeptides from human glycophorin A that bore three NeuAcα2–3Galβ1–3(NeuAcα2–6)GalNAc serine/threonine-linked tetrasaccharide chains and their sequentially deglycosylated derivatives were also applied to this column. Glycopeptides carrying three constitutive GalNAc-Ser/Thr(Tn-antigen) had the strongest affinity, whereas those with fully sialylated carbohydrate tetrasaccharide chains showed weak interaction. The association kinetics of Asn-linked glycopeptides from bovine asialofetuin to recombinant macrophage lectin was determined by surface plasmon resonance spectroscopy. The results indicated k_{assoc} value of 1.63×10^4 M^{-1} s^{-1}. The calculated value for K_a was 6.20×10^7 M.

Members of a calcium-dependent animal lectin family (so-called C-type lectins) are involved in a variety of cellular recognition and adhesion events (Drickamer, 1988; Hoyle & Hill, 1988; Lasky et al., 1989; Bevilacqua et al., 1989; Johnston et al., 1989). Galactose/*N*-acetylgalactosamine-specific C-type lectins (MMGL)[1] were previously found on the surface of hepatocytes and macrophages (Mϕ)(Ii et al., 1990; Ozaki et al., 1992, 1993; Imamura et al., 1984; Oda et al., 1988, 1989; Sato et al., 1992). They apparently mediate endocytosis of asialoglycoproteins (Ozaki et al., 1990, 1993). On activated Mϕ these molecules also seemed to be involved in the recognition of tumor cells (Imamura et al., 1984; Oda et al., 1988, 1989; Sato et al., 1992), although the molecular and cellular basis for the recognition was not well-understood. Levels of mRNA of MMGL were higher in thioglycolate-induced inflammatory Mϕ and in OK432-elicited tumoricidal Mϕ than in resident Mϕ (Sato et al., 1992). MMGL contains a carbohydrate recognition domain (CRD) in its carboxy-terminal portion and a transmembrane region near its amino-terminal portion to form a type 2 membrane protein (Drickamer, 1988). The C-type CRD was originally defined as a proteolytic fragment from hepatic lectins. These fragments showed Ca^{2+}-dependent carbohydrate-binding activity (Drickamer et al., 1986). This region contains a sequence motif of approximately 30 conserved amino acids spreading over about 120 amino acids (Drickamer, 1988). The family members include endocytic receptors such as hepatic lectins (asialoglycoprotein receptors) (Ashwell & Harford, 1982), selectins, which are cell adhesion molecules expressed on activated endothelial cells, activated platelets, and lymphocytes (Lasky et al., 1989; Bevilacqua et al., 1989; Johnston et al., 1989), proteoglycan core proteins (Halberg et al., 1988), low-affinity receptors for IgE (Kikutani et al., 1986), NKR-P1, a signal transduction molecule on natural killer cells (Giorda et al., 1990), and pulmonary surfactant apoproteins (White et al., 1985). Although each of these proteins contains Ca^{2+}-dependent CRD, it displays distinct carbohydrate-binding specificity as defined by their interaction with monosaccharides. In the present study, we have investigated the carbohydrate-binding specificity of MMGL by means of affinity chromatography using immobilized recombinant MMGL (rML). The results from the comparison of a series of oligosaccharides and glycopeptides revealed that rML recognized a group of carbohydrate chains often expressed on the surface of a variety of malignant cells. Because a biosensor based on surface plasmon resonance (SPR) has successfully been used for the binding analysis of recognition molecules

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• Abstract published in *Advance ACS Abstracts*, June 1, 1994.

[1] Abbreviations: CRD, carbohydrate recognition domain; HBS, 10 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 0.05% BIAcore surfactant P20 in distilled H_2O; MMGL, mouse macrophage galactose/*N*-acetylgalactosamine-specific C-type lectins; Mϕ, macrophage; rML, recombinant MMGL; RU, resonance unit; SPR, surface plasmon resonance; TBS, 50 mM Tris-HCl, pH 8.0, containing 0.15 M NaCl.

0006-2960/94/0433-8159$04.50/0

(Schuster et al., 1993), one of the carbohydrate chains having affinity with rML was further analyzed for its association with rML by this technique.

EXPERIMENTAL PROCEDURES

Production and Isolation of Soluble rML. cDNA encoding the extracellular region of MMGL flanked by artificial sites for *Nco*I and *Bam*HI was amplified by the polymerase chain reaction using primers 5'GGCCATGGACCTAGGCACCCTG3' and 5'CGGATCCTAGCTCTCCTTGGCC3' as described previously (Sato et al., 1992). The polymerase chain reaction generated DNA was digested with *Nco*I and *Bam*HI and inserted between the *Nco*I and *Bam*HI sites of expression vector pET-3d to yield a plasmid pET-3dMfL. The constructed plasmid was introduced into *Escherichia coli* strain BL21(DE3) cells. The BL21(DE3) cells containing the plasmid pET-3dMfL were grown to mid log-phase at 37 °C in LB medium and then treated with isopropyl β-D-thiogalactoside at a concentration of 1 mM. After isopropyl β-D-thiogalactoside induction, the cultured cells were washed with 50 mM Tris-HCl, pH 8.0, containing 0.15 M NaCl (TBS) and suspended in TBS containing 1 mM phenylmethanesulfonyl fluoride. The cell lysates were prepared by freezing and thawing, and after the addition of 20 μg/mL DNase I, centrifuged at 15000*g* for 10 min at 4 °C. The pellet was washed with TBS containing 0.5% Triton X-100 and 10 mM EDTA and then with H_2O. The washed pellets were solubilized with 2 M NH_4OH and then dialyzed against 25 mM sodium maleate buffer, pH 6.2, containing 20 mM $CaCl_2$, 0.5 M NaCl, 1 mM phenylmethanesulfonyl fluoride, and 1 mM glutathione at 4 °C. rML was purified by affinity chromatography on a column of lactose–Sepharose as described previously (Sato et al., 1992). The product had an approximate M_r 24 000 as demonstrated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. In aqueous buffers, its apparent size corresponded to the eluting position of M_r 150 000 on Sepharose CL4B.

rML Affinity Chromatography. The purified rML was coupled to formylcellulofine (Seikagaku Kogyo, Tokyo, Japan) according to the method of Jentoft and Dearborn (1979) at a concentration of 5 mg/mL gels. Affinity chromatography was performed at 22 °C. A radiolabeled oligosaccharide or glycopeptide samples (2000 cpm in 100 μL) were loaded onto a column of rML–cellulofine (0.6 × 4.0 cm) equilibrated with 50 mM Tris-HCl, pH 6.8, containing 10 mM $CaCl_2$. The column was eluted with the same buffer and then with 50 mM Tris-HCl, pH 6.8, containing 10 mM EDTA. Fractions (0.2 mL) were collected at a flow rate of 0.6 mL/h. The radioactivity of each fraction was measured on a liquid scintillation counter.

Glycopeptides and Oligosaccharides Used for Affinity Chromatography. The structures of the glycopeptides and oligosaccharides used in this study are shown in Figure 1. They were prepared as previously described, and their structures were confirmed by compositional analyses, methylation analyses, and sequential glycosidase digestions (Sueyoshi et al., 1988a,b). Various Asn-linked glycopeptides were prepared by repeated Pronase digestion of the corresponding glycoproteins (Tsuji et al., 1981; Yamamoto et al., 1981). Oligosaccharides were released from glycopeptides by hydrazinolysis as described previously (Tsuji et al., 1981; Yamamoto et al., 1981). Fractionation of Asn-linked oligosaccharides was generally carried out as in the following example for transferrin oligosaccharides: Asn-linked complex-type oligosaccharides from human serum transferrin were applied to a column (1.0 × 5.0 cm) of Con A–Sepharose (Pharmacia) equilibrated with 0.05 M sodium acetate buffer, pH 6.0, containing 0.15 M NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$. After elution of triantennary oligosaccharides with the same buffer, biantennary oligosaccharides that had been retained by the column were eluted with the same buffer containing 0.1 M methyl α-D-mannoside. After removal of sialic acid residues by mild acid hydrolysis (0.1 M HCl, 80 °C, 30 min), the Con A-unbound fraction was loaded on an L-PHA-Sepharose column (0.5 × 12.0 cm). The column was eluted with 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. The structures of L-PHA-retarded and unretarded fractions were triantennary oligosaccharides with an outer α-mannosyl residue substituted at C-2 and C-6 and similar oligosaccharides with C-2 and C-4, respectively (Cummings, 1982). The Con A-bound fraction was further fractionated by anion-exchange chromatography on a column (1.5 × 16.0 cm) of DEAE-Sephadex A-25. The column was eluted with 2 mM Tris-HCl, pH 7.4, and then with a linear gradient of NaCl (0–0.2 M) in the same buffer, to yield biantennary monosialo- and disialo-oligosaccharides. Their carbohydrate structures were confirmed by gel permeation chromatography on Bio-Gel P-4 before and after sequential removal of nonreducing terminal saccharides.

Hybrid-type glycopeptide GP-I from ovalbumin was obtained according to the method of Yamashita et al. (1978). An asialo-tetraantennary oligosaccharide from human α1-acid glycoprotein was prepared according to the method of Yoshima et al. (1981). Tritiated *N*-acetyllactosamine was synthesized as follows: UDP-[4,5-^{3}H] Gal (30 Ci/mmol; New England Nuclear, Boston, MA) and GlcNAc were incubated with 0.1 unit of β1–4 galactosyltransferase in 0.1 mL of 10 mM HEPES, pH 8.0, containing 0.15 M NaCl at 37 °C for 2 h. ^{3}H-Labeled *N*-acetyllactosamine was purified on the column of Bio-Gel P-4 as described previously (Irimura et al., 1981). Tritiated Galβ1-3GalNAc was prepared as follows: asialo CB-II glycopeptide (described below) was radioactively labeled by the galactose oxidase–NaB^3H_4 method as described previously (Sueyoshi et al., 1988b). The asialo [^{3}H]CB-II was digested with *O*-glycanase from *Diplococcus pneumoniae* (Genzyme, Boston, MA) in 10 mM phosphate buffer, pH 6.0, at 37 °C for 18 h, and released labeled oligosaccharides were recovered from the pass-through fraction on columns of Dowex 50W-X8 and Bio-Rad AG1-X8 eluted with distilled water. Specific activities of radiolabeled glycopeptides and oligosaccharides were about 7.5 × 10^{10} and 8.9 × 10^{10} dpm/mmol, respectively.

An N-terminal glycopeptide of human erythrocyte glycophorin A (CB-II) was prepared from tryptic fragment T1 according to the methods of Prohaska et al. (1981) by cyanogen bromide cleavage and acetylated with [^{14}C]acetic anhydride (2 mCi/mmol, New England Nuclear). Sialic acid residues of [^{14}C]CB-II were removed by mild acid hydrolysis in 50 mM HCl at 80 °C for 1 h. Asialo, agalacto-[^{14}C]CB-II was obtained after Smith periodate degradation of asialo-[^{14}C]-CB-II (Irimura et al., 1981). The second-round Smith periodate degradation of asialo, agalacto-[^{14}C]CB-II gave [^{14}C]CB-II peptide without sugar moieties. The α2–3-linked sialic acid residues of [^{14}C]CB-II were removed by α2–3 sialidase from *Salmonella typhimurium* (Sigma, St. Louis, MO). Removal of the sialic acid residues was confirmed by the eluting position of the anion-exchange chromatography on Mono Q column (Pharmacia, Sweden). The structures of the derivatives of CB-II are summarized in Figure 2.

Preparation of Glycopeptides from Asialofetuin and Their Immobilization on a Gold Membrane. One milligram of asialofetuin (Sigma) was dissolved in 890 μL of a mixture of

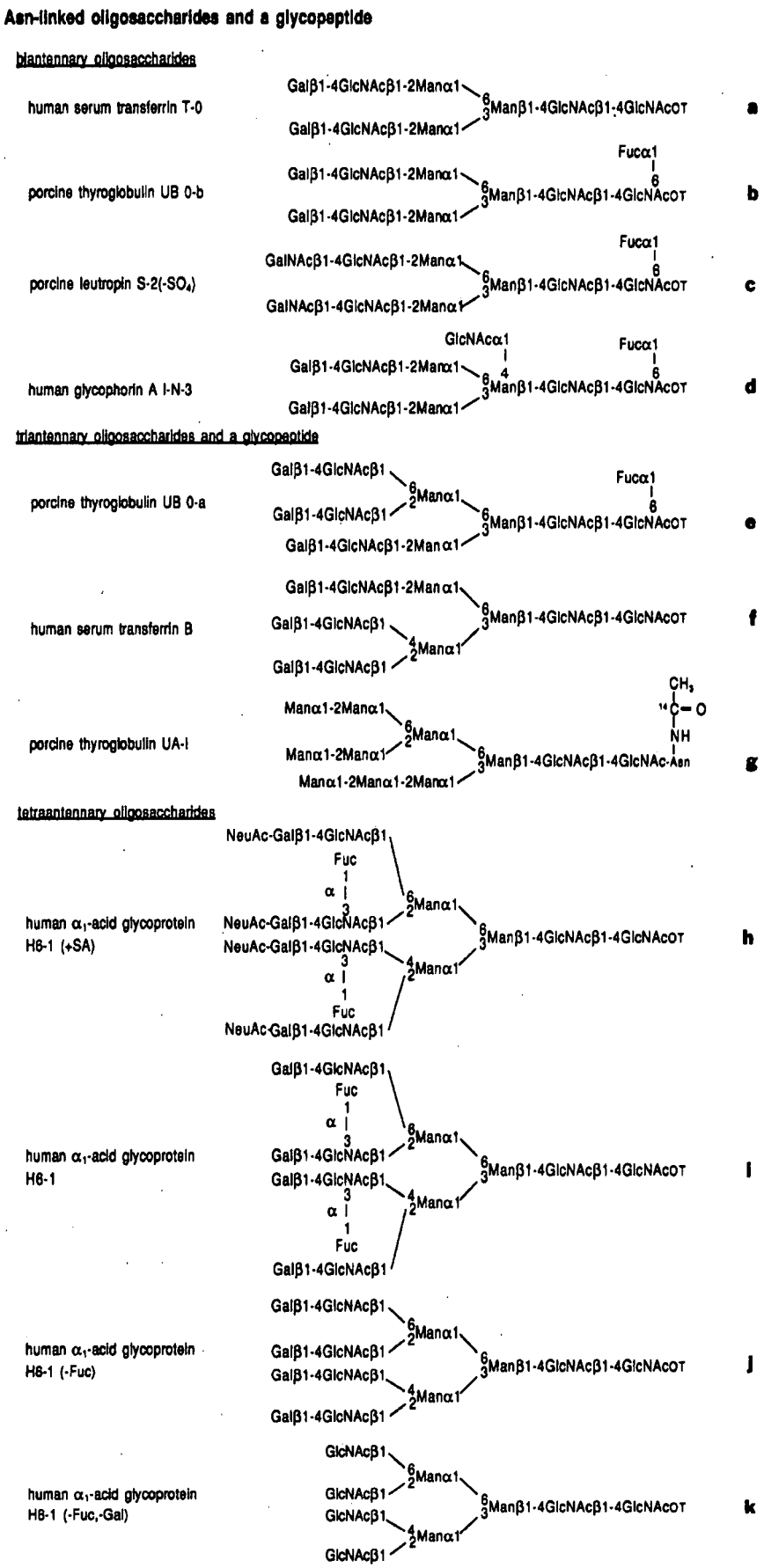

FIGURE 1: Structures of N-linked oligosaccharides used in this study. Details are described under Experimental Procedures.

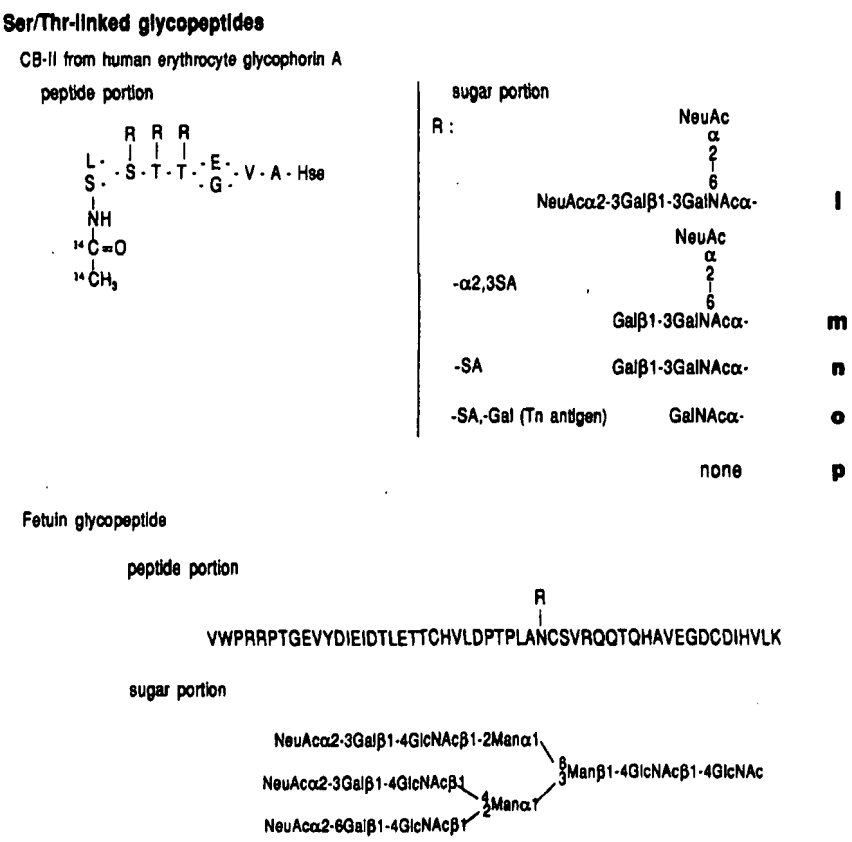

FIGURE 2: Structures of glycopeptides used in this study. Octapeptides derived from the amino-terminal portion of human glycophorin A (CB-II) were subjected to sequential chemical and enzymatic trimming of carbohydrate chains. The amino acid sequence is based on that of Marchesi et al. (1976). Asialofetuin glycopeptides were immobilized on the surface of the sensor tip.

6 M guanidine hydrochloride/0.25 M Tris/1 mM EDTA reduced with dithiothreitol according to the method of Cavins and Friedman (1970) and digested with lysylendopeptidase (Wako Pure Chemicals, Osaka, Japan) at 37 °C for 15 h in 80 μL of 0.1 M Tris-HCl buffer, pH 9.0. The molar ratio of enzyme to substrate was 1:200. Peptides were separated with SMART System (Pharmacia Biotech AB) on a μ RPC C2/C18SC 2.1/10 column. The peptides were eluted in a gradient of acetonitrile (1–80% for 51 min) in 0.065–0.05% trifluoroacetic acid at a flow rate of 100 μL/min. Peaks were detected using a μ peak monitor, set at 215, 254, and 280 nm. Each peak was tested for the presence of carbohydrate chains by affinity chromatography with immobilized *Ricinus communis* agglutinin. The lysylendopeptidase digest of asialofetuin gave two glycopeptides (peaks 1 and 2). Amino acid sequences of these glycopeptides were determined with a Shimadzu amino acid sequencer (Model PPSQ-10). Judging from the complete amino acid sequence and the structures of the three N-linked glycosylation sites of fetuin (Dziegielewska et al., 1990), peak 1 contained one N-linked carbohydrate chain and peak 2 contained two N-linked carbohydrate chains. From the study by Dziegielewska and co-workers, glycopeptides in peak 1 should mainly consist of trianntennary oligosaccharides as indicated in Figure 2 (Yet et al., 1988). This fraction was pooled and used for the analysis of glycopeptide–rML interaction after being immobilized on gold membranes (see below).

Biospecific Interaction Analysis Using BIAcore Sensor. BIAcore (Pharmacia Biosensor AB, Uppsala, Sweden) is useful to analyze the nature of specific molecular interactions on the principle of surface plasmon resonance (SPR) (Karlsson et al., 1991; Liedburg et al., 1983; Lofas & Johnson, 1990; Fagerstam et al., 1992). SPR is a phenomenon that occurs between incoming photons and the electrons in the surface of a thin gold film coated onto a glass support of the sensor chip CM5 (Lofas & Johnson, 1990; Fagerstam et al., 1992). At the specific wavelength and angle of incident light, energy is transferred to the electrons in the metal surface, causing the reflected light to disappear. At this angle of nonreflectance, the refractive index corresponds to the mass on the surface of the gold film. The changes in the mass concentration that BIAcore measures are those due to the binding and dissociation of the interacting molecules. Onto this gold film is linked a carboxymethylated dextran, which is used for immobilization of glycopeptides.

HEPES-buffered saline (HBS) used for SPR measurement was prepared from 10 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, and 0.05% BIAcore surfactant P20 (Pharmacia Biosensor) in distilled H_2O. A Ca^{2+}-free buffer with 10 mM EDTA instead of 1 mM $CaCl_2$ was used to test the Ca^{2+} dependency of the interaction between rML and glycopeptides. All buffers used were filtered (0.2 μm) and thoroughly degassed prior to use. The surface of the sensor chip of BIAcore was activated by injection of a mixture of 100 mM *N*-hydroxysuccinimide and 400 mM *N*-ethyl-*N'*-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (35 μL) into the flow cell. Glycopeptides dissolved in acetate buffer, (pH 5.0), were injected into the activated surface at a flow rate of 5 μL/min. Excess unreacted *N*-hydroxysuccinimide esters were deactivated by reacting with a large excess (35 μL) of 1 M ethanolamine (adjusted at pH 8.5 with NaOH). A final washing with 0.1 M H_3PO_4 was performed to remove nonspecifically bound molecules. The difference between the baseline of SPR response before and after the immobilization

of glycopeptides corresponded to the amount of immobilized glycopeptides. The same glycopeptide-coated surface was used repeatedly to study lectin samples under various conditions. This was accomplished by removing the associated lectin with 0.1 M H_3PO_4.

Kinetic Measurement of Glycopeptide–Mϕ Lectin Interactions. For kinetic measurement, rML at various concentrations (15.6–250 μg/mL) in HBS sample buffer was injected over the glycopeptide-immobilized surface at a flow rate of 2 μL/min. The temperature was maintained at 25 °C during the binding analyses. The association rate for binding between asialofetuin glycopeptide (peak 1) and rML was expressed by

$$dR/dt = -(k_{assoc}C + k_{diss})R + k_{assoc}CR_{max}$$

where k_{assoc} is the association rate constant, k_{diss} is the dissociation rate constant, R_{max} is the maximum binding capacity (in RU) of the immobilized glycopeptide surface, R is the amount of bound rML measured by the SPR response (RU) at a given time (t) (Fagerstam et al., 1992), and C is the constant concentration of rML injected into the glycopeptide-coated surfaces. A linear plot of dR/dt *vs* R yielded the following:

$$\text{slope} = -(k_{assoc}C + k_{diss})$$

dR/dt was obtained from measurements of the slope at various time points along the real time association curve. By plotting the slopes of the dR/dt *vs* R lines as a function of rML concentration C, a new line was obtained. Using this plot, the association rate constant (k_{assoc}) and the dissociation rate constant (k_{diss}) were calculated from the slope and the y intercept, respectively.

RESULTS

Affinity Chromatography of Glycopeptides with N-Linked Carbohydrate Chains on Immobilized rML. To elucidate the carbohydate-binding specificity of MMGL, affinity chromatography on an immobilized rML column was performed at 22 °C. Retardation from this column was observed with several different radiolabeled glycopeptides and oligosaccharides. First, complex-type, high mannose-type, and hybrid-type glycopeptides were compared for their interaction with rML columns. Figure 3 shows the elution profiles of desialized biantennary, triantennary, and tetraantennary complex-type glycopeptides prepared as described under Experimental Procedures. The degree of retention of these glycopeptides increased in parallel with the number of nonreducing terminal Galβ1–4GlcNAc structures in these glycopeptides. High mannose-type, hybrid-type, or asialo–biantennary complex-type glycopeptides did not show retardation on rML columns.

Tetrasialyl complex-type glycopeptide H6–1(+SA) and its sequential degradation products were tested as to binding to rML columns (Figure 4). As expected, fully sialylated glycopeptide did not show any affinity. After sialidase treatment, the resultant glycopeptide (H6-1) showed strong affinity with rML columns. Digestion of this glycopeptide with fucosidase did not affect the elution profile. However, removal of terminal galactosyl groups from H6-1 markedly decreased the affinity of this glycopeptide with rML columns. Therefore, β-galactosyl residues at nonreducing termini seem to be essential for complex-type Asn-linked sugar chains to interact with rML. The relative strength of the affinity as estimated from the degree of retardation increased according to the number of terminal galactosyl groups. Retardation of galactosylated complex-type oligosaccharides was not seen

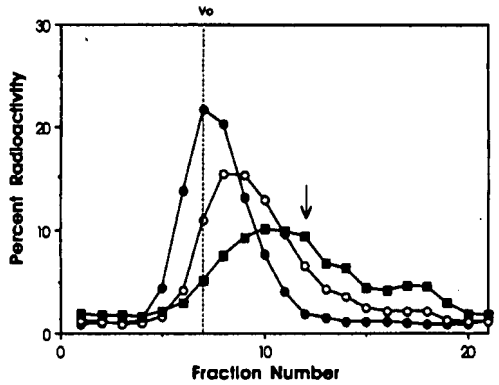

FIGURE 3: Elution profiles of Asn-linked oligosaccharides or glycopeptides on immobilized rML column. (Solid circle) Asialo-biantennary complex-type carbohydrate chains from human transferrin (oligosaccharide a). Other asialo-biantennary complex-type carbohydrate chains (oligosaccharides b, c, and d) eluted at positions similar to that of oligosaccharide a. (Open circle) Asialo–triantennary complex-type carbohydrate chains from porcine thyroglobulin (oligosaccharide e); another asialo–triantennary complex-type carbohydrate chains (oligosaccharide f) eluted at a position similar to that of oligosaccharide e. (Solid square) Fucosylated asialo–tetraantennary complex-type chains from human α1 acid glycoprotein (oligosaccharide i). Arrow indicates the eluting position where the elution buffer was switched to a buffer containing 10 mM EDTA.

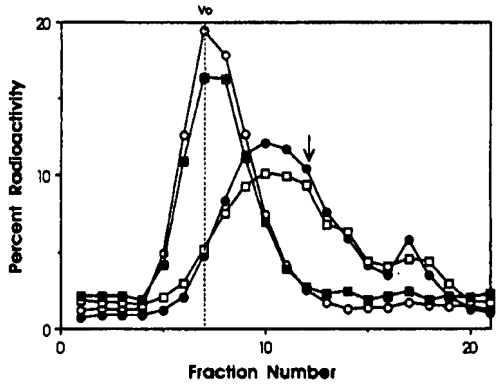

FIGURE 4: Elution profiles of Asn-linked tetraantennary complex-type glycopeptide H6-1 and its derivatives on immobilized rML column. (Open circle) Sialyated and fucosylated tetraantennary complex-type carbohydrate chains (oligosaccharide h). (Solid circle) Fucosylated asialo–tetraantennary complex-type carbohydrate chains (oligosaccharide i). (Open square) Asialo-tetraantennary complex-type carbohydrate chains without fucose (oligosaccharide j). (Solid square) Asialo–agalacto–tetraantennary complex-tpye carbohydrate chains without fucose (oligosaccharide k). Arrow indicates the eluting position where the elution buffer was switched to a buffer containing 10 mM EDTA.

when 10 mM EDTA was added to the elution buffer, indicating that the interaction was calcium dependent. Interaction of oligosaccharides with the rML column was not seen when 0.1 M galactose was added to the elution buffer. The interaction was apparently stronger at 4 °C than at 22 °C. Difference in the elution profiles was not detected at pH 6.0, 6.5, 7.0, and 7.5.

Affinity Chromatography of Glycopeptides with O-Linked Carbohydrate Chains on Immobilized rML. Figure 5 shows the elution profiles of glycopeptide CB-II and digestion products from CB-II. [^{14}C]CB-II, obtained by cyanogen bromide cleavage of human glycophorin A, constitutes the N terminus of this glycoprotein and contains O-linked tetrasaccharides. Intact [^{14}C]CB-II eluted at the void volume fraction from rML columns. After removal of single sialic acid attached to galactosyl residues in each tetrasaccharide, CB-II was only slightly retarded from rML columns. However,

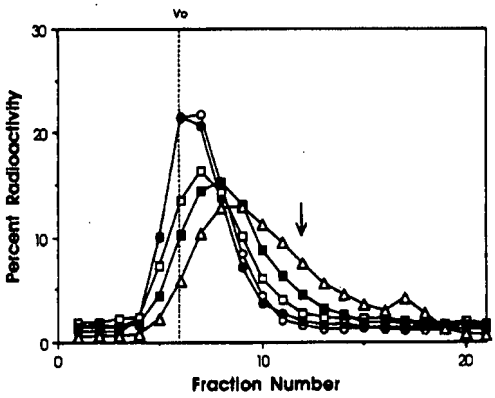

FIGURE 5: Elution profiles of glycopeptide CB-II and its derivatives on immobilized rML column. (Open circle) CB-II with intact carbohydrate chains (glycopeptide l). (Open square) CB-II after removal of α2,3-linked sialyl residues (glycopeptide m). (Solid square) CB-II after removal of all sialyl residues (glycopeptide n). (Open triangle) CB-II after removal of all sialyl and galactosyl residues (glycopeptide o). (Solid circle) CB-II after removal of all carbohydrate residues (glycopeptide p). Arrow indicates the eluting position where the elution buffer was switched to a buffer containing 10 mM EDTA.

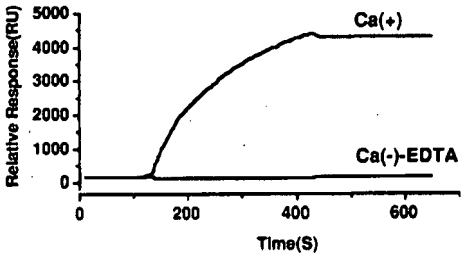

FIGURE 6: Sensorgrams showing the calcium dependency of rML–glycopeptide binding. rML was injected onto sensor chip in the presence or absence of Ca^{2+}.

completely desialylated [^{14}C]CB-II retarded from these columns. When the galactosyl groups were removed from desialized [^{14}C]CB-II by Smith degradation, the resultant glycopeptides strongly interacted with rML columns. Deglycosylated [^{14}C]CB-II peptide did not show interaction with rML. Asialo, agalacto-[^{14}C]CB-II did not show retardation on these columns when 0.1 M galactose or 10 mM EDTA was added to the elution buffer, indicating that Galβ1-3GalNAc and GalNAc moieties of [^{14}C]CB-II interacted with rML columns.

Measurement of Association of rML with Asialofetuin Glycopeptides with SPR. When a lectin was introduced onto the gold membrane surface coated with asialofetuin glycopeptides, a change in the resonance angle occurred. This was apparently due to the increase in the mass on the surface by the interaction of lectins with glycopeptides. Through BIAcore software, the resonance angle was presented in resonance units (RU). A response of 1000 RU corresponded to a shift of 0.1° in the response angle, which in turn represents a change in surface protein concentration of ~1 ng/mm^2. rML (100 μg/mL) in HBS without or with calcium ion (1 mM) was injected over the glycopeptide-immobilized surface at a flow rate of 2 μL/min. An increase in RU within 400 s was observed in the presence of Ca^{2+} (Figure 6). In the presence of 10 mM EDTA, RU did not change. The change in the response, therefore, was apparently due to interaction of rML with asialofetuin glycopeptides. When 0.1 M galactose, an inhibitory sugar for binding to rML, was co-injected with rML into the surface, no significant increase in SPR was detected (data not shown). This indicates that the interaction between rML and glycopeptide is sugar specific.

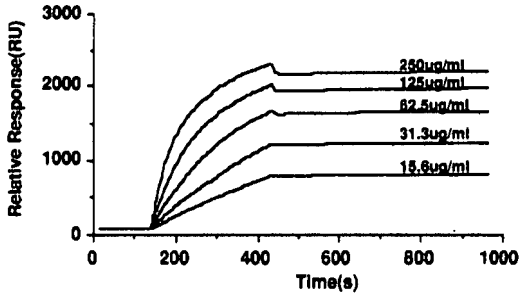

FIGURE 7: Sensorgrams showing the interaction of rML with immobilized glycopeptide. rML (10 μL) at concentrations ranging from 15.6 to 250 μg/mL in HBS was injected onto sensor chip.

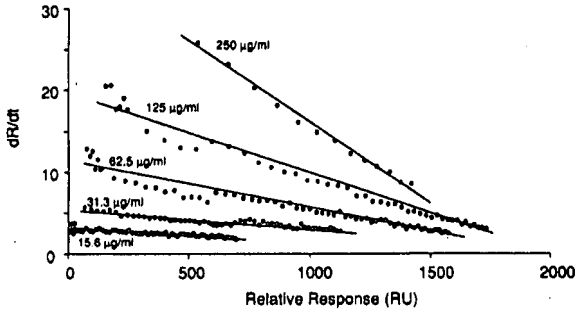

FIGURE 8: Plots of dR/dt against relative response for the interaction at rML concentrations from 15.6 (leftmost line) to 250 μg/mL (rightmost line).

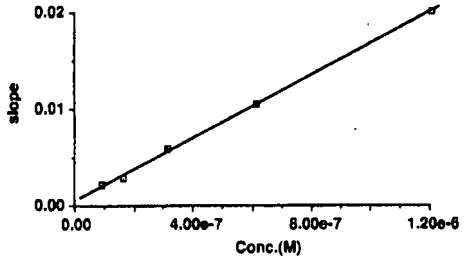

FIGURE 9: Slope of dR/dt *vs* relative response for the interaction, plotted against rML concentration. The slope of this curve gives k_{assoc}. Data are from Figure 8.

Kinetic Measurement of Glycopeptide–Mϕ Lectin Interactions. To estimate the kinetic parameters, rML at various concentrations was repeatedly applied to the glycopeptide-coated surfaces. The data are summarized in Figure 7. Following rML injections, the SPR response increased gradually in a concentration-dependent manner. At 420 s after the initial sample injection, rML pulse was replaced by HBS buffer. The response curve after 420 s reflected the dissociation rate, which was very slow. dR/dt values were obtianed beginning at 30 s after the injection, to avoid an initial fast change in bulk solution refractive index, and continued until the interaction approached a steady state. During this period, the dR/dt *vs* R plots were linear (Figure 8). The slope of each line was plotted against the concentration of injected rML, which was also linear (Figure 9), allowing accurate estimation of the association rate constnat (k_{assoc}) from the slope (1.6×10^4 M^{-1} s^{-1}). The dissociation rate constant (k_{diss}) was also estimated from the y intercept of the same figure as 2.6×10^{-4} s^{-1}. The affinity constant, K_a, was then calculated from k_{assoc}/k_{diss} to be 6.2×10^7 M. The amount of immobilized glycopeptide (1710 and 556 RU) had little effect on the kinetic parameters.

DISCUSSION

Mϕ express a variety of carbohydrate-binding molecules on their surfaces. These lectins are thought to have different functions expressed on distinct Mϕ subpopulations. For example, Hill and co-workers (Haltiwanger and Hill, 1986) isolated lectins from rat alveolar Mϕ. These lectins were apparently specific for terminal mannose and fucose. Lectins specific for galactose and *N*-acetylgalactosamine were previously purified from activated Mϕ obtained from ascitic cells (Oda et al., 1988, 1989). However, the precise carbohydrate-binding specificity was not previously investigated due to the limitation of the amount of available materials.

We prepared affinity chromatography adsorbent with rML to overcome this difficulty. Various carbohydrate chains with radioactive tracers were tested for their elution profiles on this column absorbent. It was shown that a cluster of terminal galactose residues or *N*-acetylgalactosamine residues had high affinity with this lectin. In the case of serine/threonine-linked carbohydrate chains, clusters of truncated forms known as Thomsen-Friedenreich antigen and Tn antigen had high affinity. In the case of asparagine-linked carbohydrate chains, tetraantennary complex-type carbohydrate chains with nonreducing terminal galactose residues had the highest affinity among various carbohydrate chains tested. Lee et al. (1983) reported that rabbit hepatic lectin had 50-fold higher affinity with triantennary complex-type oligosaccharides having 2,4-branched mannose than with triantennary isomer having 2,6-branched mannose. MMGL, however, did not distinguish the branching pattern as to the *N*-acetyllactosamine sequence at an outer mannose residue. This may be due to the homohexamer subunit structure of MMGL as compared to heterohexamer structure with two distinct subunits of hepatic galactose/*N*-acetylgalactosamine-specific lectins. As described under Experimental Procedures, our rML preparation apparently consisted of a hexamer and lacked the transmembrane domain.

This C-type lectin might be able to distinguish malignant cells from normal cells as judged from the binding on purified ^{125}I-labeled native MMGL to various normal and malignant cells (Oda et al., 1989). These results indicated that the carbohydrate specificity of MMGL was somewhat promiscuous, and MMGL showed a strong tendency to interact with carbohydrate chains characteristics to malignant cells. Tn-antigen is known to be expressed on a variety of carcinoma cells (Springer, 1989; Øntoft et al., 1990). Furthermore, highly branched complex-type oligosaccharides are thought to be specific for transformed and tumorigenic cells (Warren et al., 1978). It remains to be elucidated whether tumor cells with carbohydrate chains having affinity with MMGL show distinct survival and malignant behavior *in vitro* and *in vivo*.

In the binding study, a biosensor was successfully used for kinetic analysis. The k_{assoc}, k_{diss}, and K_a obtained for the interaction between rML and asialo–triantennary complex-type carbohydrate chain were 1.6×10^4 M^{-1} s^{-1}, 2.6×10^{-4} s^{-1}, and 6.2×10^7 M, respectively. Although k_{diss} can also be obtained from the dissociation phase (Karlsson et al., 1991), the extremly slow dissociation of rML and glycopeptide in this study makes such effort difficult. Since we cannot measure k_{diss} when it is smaller than ca. 10^{-5} s^{-1} in the present system, the k_{diss} values obtained in this study might be overestimates. The K_a value between rML and asialo–triantennary complex-type carbohydrate chains was similar to the value reported for other mammalian lectins (10^7–10^8 M^{-1}) by Scatchard analysis (Taylor & Summerfield, 1987; Lennartz et al., 1987; Siripont et al., 1988). The K_a value was also comparable to the value obtained for *R. communis* agglutinin I (RCA-I), which bound glycopeptides from asialofetuin. The k_{assoc} and k_{diss} values obtained by the kinetic analysis of rML with asialofetuin glycopeptides were different from the values of RCA-I, where k_{assoc} and k_{diss} of rML were 20 and 7 times lower, respectively. Judging from the relative eluting positions of various glycopeptides and oligosaccharides on immobilized rML, K_a of asialo–tetraantennary complex-type carbohydrate chains and asialo- and asialoagalacto-CB-II with MMGL should be greater than that of asialo–triantennary complex-type carbohydrate chains from fetuin. There seems to be a descrepancy between high affinity of rML binding to immobilized glycopeptides and moderate affinity of oligosaccharides and glycopeptides to immobilized rML. The reason for this difference was not clear. One possibility might be related to the distinction between the nature of the interaction of a multivalent ligand with a solid-phase bound multivalent receptor. The apparent high affinity might be caused by the multipotential interaction. This and other possibilities should be further examined. Nonetheless, high K_a and k_{assoc} values may contribute to rapid adhesion of Mϕ to target cells. It had been postulated that cell-cell interaction through carbohydrate–protein interactions occur as an initial step during the cascade of cell attachment and migration. Therefore, MMGL may play a significant role in the specific recognition of malignant cells by activated Mϕ.

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α-*N*-Acetylgalactosamine-capping of chondroitin sulfate core region oligosaccharides primed on xylosides

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We previously reported that cultured mammalian cells incubated with 4-methylumbelliferyl (MU) or *p*-nitrophenyl (pNP) β-xyloside synthesize an α-GalNAc-terminated pentasaccharide resembling the glycosaminoglycan-core protein linkage region. Here we show that human melanoma M21 cells and human neuroblastoma cells incubated with Xylβ-MU/pNP also make an α-GalNAc-terminated heptasaccharide containing one chondroitin disaccharide repeat. High performance liquid chromatography and matrix-assisted laser desorption ionization mass spectrometry analysis of intact or glycosidase-digested xyloside showed the structure as: GalNAcαGlcAβ1,3GalNAcβ1,4GlcAβ1,3Galβ1,3Galβ1,4Xylβ-MU/pNP. The α-GalNAc-terminated xylosides can account for ~10% of the total Xylβ-MU/pNP products (~1.5 nmol/h/mg). These results show that GalNAcαGlcAβ-modification is relatively abundant, but not unique to the GAG-linkage tetrasaccharide. α-GalNAc addition to the GlcA residue does not appear to be an extension of general phase II detoxification of xenobiotics that involve glucuronidation, since M21 cells incubated with MU synthesize only 0.3 pmol GlcAβ-MU/h/mg protein, and undetectable amount of GalNAcαGlcAβ-MU (<40 fmol/h/mg). Further, subcellular fractionation shows that the α-*N*-acetylgalactosaminyltransferase activity colocalizes in the Golgi with other glycosyl transferases and not in the ER, where xenobiotic detoxification glucuronosyltransferases are found. Although GalNAcαGlcAβ-terminal modification has not been detected on naturally occurring GAG chains, the substantial amount of α-GalNAc transferase activity suggests that the α-GalNAc transferase could utilize other GlcA-containing glycoconjugates as acceptors.

Key words: α-*N*-acetylgalactosamine/chondroitin sulfate/glycosaminoglycans/phase II detoxification/xylosides

Introduction

Priming high molecular weight glycosaminoglycan (GAG) chains on β-D-xylosides (Xyl) is well-established, but the GAG chains comprise only a small portion of the xyloside products (Salimath *et al.*, 1995). The major ones consist of small oligosaccharides (Freeze *et al.*, 1993). Some resemble the known intermediates in GAG chain synthesis, such as the tetrasaccharide, GlcAβ1,3Galβ1,3Galβ1,4Xylβ-R that links GAG chains to protein and serves as a common precursor for both heparan (GlcNAcα1,4GlcAβ1,4)$_n$ and chondroitin (GalNAcβ1,4GlcAβ1,3)$_n$ extension. Other major products, e.g., Siaα2,3Galβ1,4Xylβ-R, have no naturally known counterpart (Freeze *et al.*, 1993; Takagaki *et al.*, 1996). Small amounts of other unusual products are also seen (Izumi *et al.*, 1994; Nakamura *et al.*, 1994; Manzi *et al.*, 1995; Shibata *et al.*, 1995). We previously showed that the major product made by melanoma cells incubated with low concentrations of 4-methylumbelliferyl (MU) or *p*-nitrophenyl (pNP) β-xylosides is GalNAcα1,4GlcAβ1,3Galβ1,3Galβ1,4Xylβ-MU/pNP (Manzi *et al.*, 1995; Salimath *et al.*, 1995). The terminal α-GalNAc has not been found on any known GAG-chain intermediates.

Recently, Kitagawa *et al.* found a novel α-*N*-acetylgalactosaminyltransferase activity in fetal bovine serum (Kitagawa *et al.*, 1995) which is most likely responsible for the production of the α-GalNAc-capped xylosides we described. It is not clear whether this type of modification is specific for carbohydrate–protein linkage region of GAG chain or if GalNAcαGlcAβ-R terminal can be found on chondroitin sulfate or heparan sulfate. In this article, we describe an additional α-GalNAc-capped heptaoligosaccharides made by human melanoma cells and neuroblastoma cells incubated with Xylβ-MU or Xylβ-pNP. The sequence of the novel xyloside was determined, showing that the α-GalNAc-terminated molecules are not restricted to the core region of GAG chain. Also, the addition of α-GalNAc to GlcA residue does not appear to be an extension or modification of glucuronidation of xenobiotic compounds, e.g., phase II detoxification.

Results

Production of the α-GalNAc-terminated xylosides and structural analysis

Human melanoma M21 cells and human neuroblastoma IMR-32 cells were labeled with [6–^{3}H]Gal in the presence of 0.5 mM Xylβ-pNP or Xylβ-MU, and the total small xyloside-primed oligosaccharides were recovered from the labeling medium. The total xylosides were analyzed on ion exchange HPLC before and after digestion with *Arthrobacter ureafaciens* neuraminidase (AUN) as shown in Figure 1, A and B, respectively. The major labeled compound was Siaα2,3Galβ1,4Xylβ-MU (major peak in Figure 1A; Freeze *et al.*, 1993). AUN converted it to Galβ1,4Xylβ-MU, but negatively charged oligosaccharides remained after the treatment (peak I and II in Figure 1B). We also found that Xylβ-pNP primed the synthesis of the same compounds in IMR-32 cells and M21 cells to much greater extent (Figure 1, C and D, respectively). As we reported previously (Manzi *et al.*, 1995; Salimath *et al.*, 1995), peak I in Figure 1B–D was identified as an α-GalNAc-capped pentasaccharide, GalNAcα1,4GlcAβ1,3Galβ1,3Galβ1,4Xylβ-MU/pNP by sequential glycosidase digestion. It accounted for 2.7–5.9% of the total label. Another oligosaccharide, peak II in Figure 1C,D seemed to possess two negative charges and was detected in all media from cells incubated with either Xylβ-MU or Xylβ-pNP. It accounted for

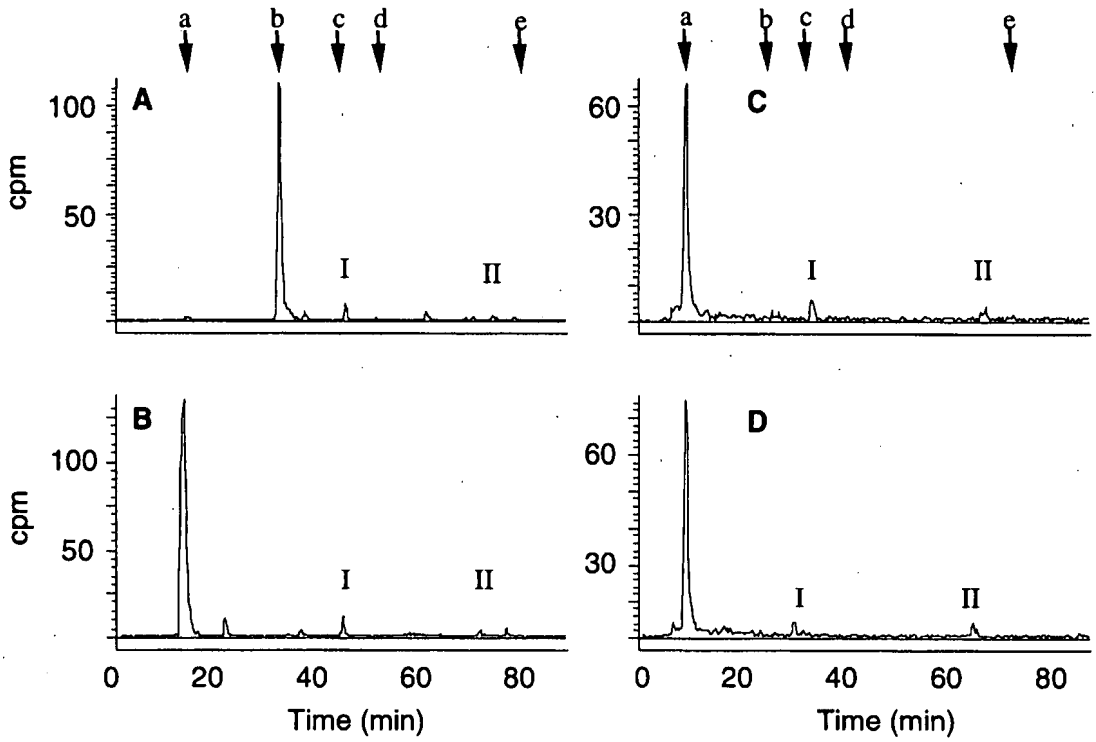

Fig. 1. Amine adsorption HPLC analysis of [6–^{3}H]Gal-labeled xylosides. (**A**) Total labeled Xylβ-MU products from M21 melanoma cells. (**B**) Sialidase (AUN) digestion of sample A. (**C**) total labeled Xylβ-pNP products from IMR-32 neuroblastoma cells. (**D**) Total labeled Xylβ-pNP products from M21 melanoma cells. Peak I and II; see text. Arrows in (**A**) and (**C**) shows authentic markers; a, Gal-Xylβ-MU/pNP; b, Sia-Gal-Xylβ-MU/pNP; c, Sia-Galβ1,3GlcNAcβ1,3Galβ1,4Glc-AMC; d, Siaα2,6Galβ1,4GlcNAcβ1,3Galβ1,4Glc-2AB; e, Siaα2,3Galβ1,3(Siaα2,6)GlcNAcβ1,3Galβ1,4Glc-2AB, where AMC is 7-amino methylcoumarin and 2AB is 2-amino benzamide.

1.5–4.5% for total recovered radioactivity, and together these two peaks can be 5–10% of the labeled xylosides.

To determine the structure of the sialidase-resistant oligosaccharide with two negative charges, the non-labeled peak II was purified from 2.5 l of culture media of M21 cells incubated with 0.5 mM Xylβ-pNP. Monosaccharide compositional analysis of 2-aminobenzamide derivatives by reversed phase HPLC showed Xyl, Gal, GlcA, and $GalNH_2$ in molar ratios of 1.0:2.3:1.7:2.0. Therefore, peak II probably contained at least the core region of the GAG chain, GlcA-Gal-Gal-Xyl-R, an additional GlcA, and two residues of (*N*-acetyl)galactosamine, suggesting the xyloside is a chondrooligosaccharide. To establish the sequence, another sample of the xyloside was sequentially digested with various exoglycosidases.

Amine adsorption HPLC was used to determine the size and the number of charges of the target material. β-*N*-Acetylhexosaminidase A (β-HexA) did not alter the elution position of the peak (Figure 2B) neither did AUN nor β-glucuronidase digestion alone. α-*N*-Acetylgalactosaminidase digestion changed the elution time, indicating that the oligosaccharide was terminated with *N*-acetylgalactosamine via α-linkage (Figure 2C). The α-*N*-acetylgalactosaminidase digested sample eluted later than the nontreated sample in spite of its smaller size. It seemed that exposing the GlcA residue in addition to the internal GlcA causes a stronger interaction with HPLC anion exchanger. The resultant peak was next digested with β-glucuronidase, leading the loss of one negative charge, but one negative charge still remained (Figure 2E). Combined digestion of peak II with α-*N*-acetylgalactosaminidase and β-glucuronidase produced same compound (Figure 2D) as in Figure 2E. The remaining oligosaccharide probably contained a GalNAc residue in β-linkage, typical of a repeating disaccharide in chondroitin. Treatment of the α-*N*-acetylgalactosaminidase-digested compound with a combination of β-glucuronidase and β-*N*-acetylhexosaminidase A neutralized the material which then coeluted with a standard of Galβ1,3Galβ1,4Xylβ-pNP, showing that it had the predicted core structure for GAG chain (Figure 2G). Further, the neutral material released free [6–^{3}H]galactose when digested with jack bean β-galactosidase (data not shown).

To confirm the identification, a portion of peak II was subjected to FAB-MS analysis in negative ion mode. The analysis showed that the parent compound has a mass of 1374 $[M\text{-}2H+Na]^-$ in agreement with the calculated mass of 1374 for GalNAcαGlcAβGalNAcβGlcAβGalβGalβXylβ-pNP (data not shown). The sample and each of the enzymatic hydrolysis products were then analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-MS). Since negative ion mode did not work well, the measurements were performed in the less sensitive positive ion mode. The parent molecule which has a mass of 1374 in FAB-MS was mostly detected as an ion $[M\text{-}H_2O+Na]^+$ of 1360. The products of the sequential exoglycosidase digestions were purified by HPLC fractionation, and analyzed by MALDI-MS. Table I shows good agreement between the estimated masses of oligosaccharides and their calculated mass expected after the corresponding glycosidase digestion.

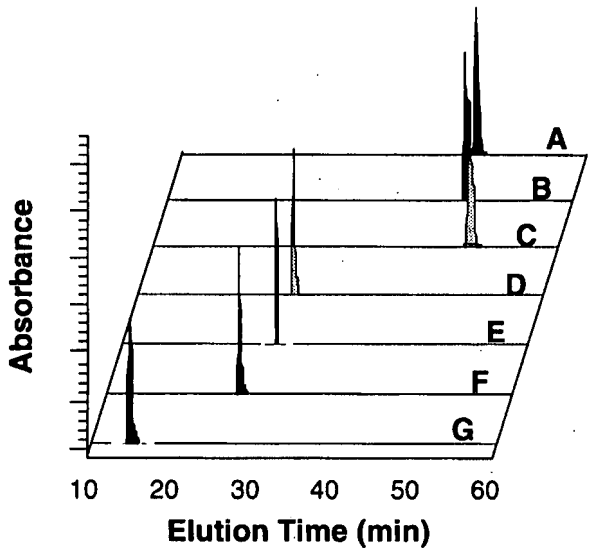

Fig. 2. Effects of glycosidase digestions on AUN-resistant Xylβ-pNP with two negative charges. (**A**) Original compound; (**B**) peak in (**A**) treated with β-*N*-acetylhexosaminidase A; (**C**) peak in (**A**) treated with α-*N*-acetylgalactosaminidase; (**D**) peak in (**C**) treated with β-glucuronidase; (**E**) peak in (**A**) treated with β-glucuronidase and α-*N*-acetylgalactosaminidase; (**F**) peak in (**D**) treated with β-*N*-acetylhexosaminidase A; (**G**) peak in (**F**) treated with β-glucuronidase.

Table I. MALDI-MS analysis of pNP-xylosides and various glycosidase-digested products

Compound in Figure 2	Molecular mass		
	Measured	Calculated	Ion
A	1361	1359	$[M\text{-}H_2O+Na]^+$
C	1158	1156	$[M\text{-}H_2O+Na]^+$
D	981	980	$[M\text{-}H_2O+Na]^+$
F	779	777	$[M\text{-}H_2O+Na]^+$
G	605	601	$[M\text{-}H_2O+Na]^+$

Table II. Specific activity of glycosyltransferases in microsomes and fractionated ER and Golgi membranes

Transferase	Microsomes	Subcellular fraction (activity, nmol/h/mg)		
		ER	Golgi I	Golgi II
GalT-I (GAG core)	0.80	0.34	1.53	1.38
β1,4GalT (LacNAc)	2.37	1.32	9.62	20.78
α-GalNAcT	0.15	0.20	1.13	0.99

Microsomes were prepared from M21 cells homogenates. Figure 4 fractions; 3, ER; 10, Golgi I; 16, Golgi II.

These results strongly suggested that the xyloside was an GalNAcαGlcAβ1,3GalNAcβ1,4GlcAβ1,3Galβ1,3Galβ1,4Xyl-β-pNP. The indicated linkage positions were not determined in this study, but are assumed based on the previously determined α-GalNAc-capped pentasaccharide and the known structure of chondroitin chains.

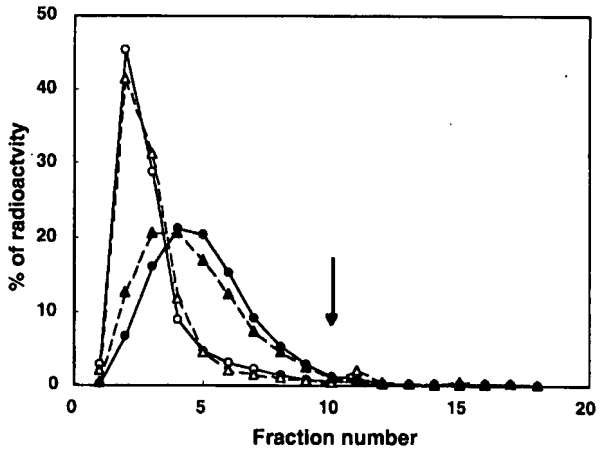

Fig. 3. *H. pomatia* agglutinin affinity chromatography of sialidase-resistant anionic [6-^{3}H]Gal-labeled Xylβ-pNP products. The [^{3}H]Gal-labeled Xylβ-pNP with either one (open circles) or two (open triangles) negative charge was applied to *H.pomatia* agglutinin column and eluted with buffer. The sample was run before (solid symbols) and after (open symbols) digestion with α-*N*-acetylgalactosaminidase. The arrow marks the starting position of 50 mM GalNAc elution.

Lectin affinity chromatography

In a previous paper, we demonstrated that an α-GalNAc-capped pentasaccharide weakly interacts with *H.pomatia* agglutinin, a lectin that recognizes terminal α-GalNAc residues including GalNAcα-Ser/Thr. The heptasaccharide was also analyzed by *H.pomatia* agglutinin affinity chromatography (Figure 3). Elution of both α-GalNAc-capped pentasaccharide and heptasaccharide was retarded compared to a nonbinding internal standard, [^{14}C]fucose. This elution is typical of weak binding. The pentasaccharide was retarded to a greater extent than the heptasaccharide probably because of its smaller size and less charge. Digestion of each with α-*N*-acetylgalactosaminidase eliminated the weak binding to the column, and they both ran in the void volume, exactly coincident with run-through [^{14}C]fucose.

GalNAcαGlcAβ is not a detoxification product

These results clearly show that GalNAcαGlcAβ-R is not restricted to the GAG chain linkage region. Viewed from another perspective this finding could mean that α-GalNAc is added to generic glucuronides, such as xenobiotic phase II detoxification products made in the ER. To answer this question, M21 cells were incubated with 1 mM 4-methylumbelliferone (MU) and after 12 h the medium was analyzed for the presence of GlcAβ-MU or GalNAcαGlcAβ-MU by amine adsorption HPLC. The medium accumulated a very low level of GlcAβ-MU (0.3 pmol/h/mg) and an undetectable level of GalNAcαGlcAβ-MU(<40 fmol/h/mg). The production of GlcAβ-MU from MU was insignificant compared to those of Siaα2,3Galβ1,4Xylβ-MU (1250 pmol/h/mg) and α-GalNAc-capped pentasaccharide (51 pmol/h/mg) when cells were incubated with 0.5 mM Xylβ-MU. Thus, it is extremely unlikely that α-GalNAc is a previously unidentified disaccharide extension product of GlcAβ-MU. In addition, M21 cells have an extremely low detoxification capacity compared to liver microsomes (Gschaidmeier *et al.*, 1995).

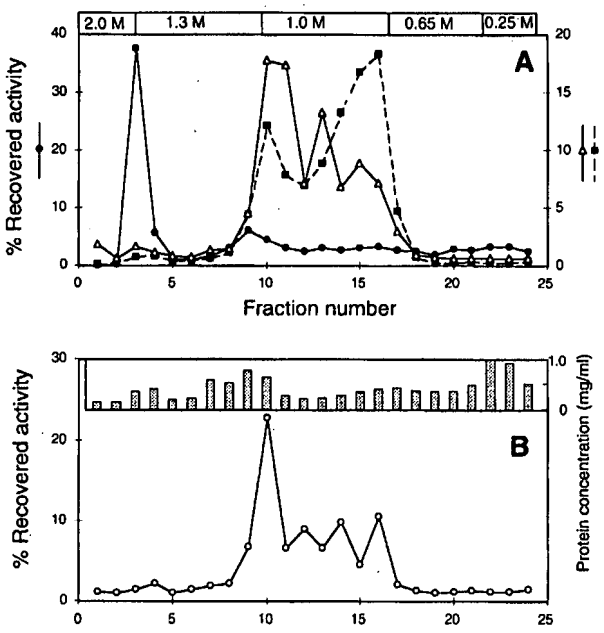

Fig. 4. Subcellular fractionation of M21 cells. M21 cells were dispersed with 20 gentle strokes in a Dounce homogenizer and a postnuclear supernatant was layered over a discontinuous sucrose gradient as shown in the bar above. After centrifugation, 0.5 ml fractions were collected from the bottom of the tube. (A) Marker enzyme α-glucosidase II was used for ER, and galactosyltransferases was used as Golgi apparatus makers; solid circles, α-glucosidase II; open triangles, GalT-I using Xylβ-pNP as an acceptor; solid circles, β1,4GalT using GlcNAcβ-Bz as an acceptor. (B) α-GalNAcT. Inset in (B) represents protein concentration of the fractions. Specific activity of each peak fraction is shown in Table II.

Although M21 cells cannot produce GalNAcαGlcAβ-MU from MU, microsomes contain a substantial amount of an α-*N*-acetylgalactosaminyltransferase (α-GalNAcT) activity that uses UDP-GalNAc as a donor and GlcAβ-MU as an acceptor (Table II). All of the GalNAc is α-linked, since it is sensitive to α-*N*-acetylgalactosaminidase digestion, but is insensitive to β-*N*-acetylhexosaminidase A digestion. Also GalNAc is linked to GlcA, since the product is no longer sensitive to β-glucuronidase (data not shown). To localize the α-GalNAcT activity, microsomes were fractionated on a discontinuous sucrose gradient into ER (α-glucosidase II) and Golgi fractions (Figure 4A) which were monitored for galactosyltransferase-I (GalT-I) used in GAG chain synthesis, and for β1,4-galactosyltransferase (β1,4GalT) used for *N*-acetyllactosamine (LacNAc) synthesis. The α-GalNAcT activity was not found in the ER fractions (fraction 3). It occurred in the Golgi membrane fractions (fraction 10–16) and substantially overlaps with GalT-I which is considered a *cis*-Golgi enzyme, but shows much less overlap with β1,4GalT which is considered a *trans*-Golgi enzyme as shown in Figure 4B. Table II shows the specific activities of α-GalNAcT and the other galactosyltransferases on fractions 3, 10, and 16 of the discontinuous sucrose gradient which were designated ER, Golgi I, and Golgi II, respectively. α-GalNAcT specific activity seemed to be comparable to that of GalT-I in Golgi I and only about 10-fold lower than β1,4GalT, one of the most abundant glycosyltransferases. Since the crude microsomal preparations contained 45–55% of the total cell proteins, the activities measured in microsomes (150 pmol/h/mg) appeared to be sufficient for the synthesis of xylosides detected in the cultured medium of M21 cells (50 pmol/h/mg). These results indicate that α-GalNAcT activity is substantial and is probably located primarily in the early and middle Golgi fractions.

Discussion

In this article, we have shown the biosynthesis of a novel xyloside-primed oligosaccharide by several cultured cells. In the previous study, we showed that several cultured cell lines incubated with Xylβ-MU/pNP produced the α-GalNAc-capped pentasaccharide, GalNAcα1,4GlcAβ1,3Galβ1,3Galβ1,4Xylβ-MU/pNP. This article extends our previous work, and demonstrates that the α-GalNAc-capping can also occur following one chondroitin sulfate disaccharide repeat.

We considered several explanations for the presence of two negative charges seen in peak II in Figure 1. Sulfate and phosphate have been found in the core linkage regions of some chondroitin sulfate chains (Fransson *et al.*, 1985; Yamada *et al.*, 1995; Moses *et al.*, 1997), but we found no evidence for those modifications. Another candidate was an elongated xyloside which carries a repeating unit of chondroitin sulfate. Monosaccharide composition analysis favored this possibility. However, the material was not susceptible to digestion with either β-glucuronidase or β-*N*-acetylhexosaminidase A. Only chicken liver α-*N*-acetylgalactosaminidase altered the elution pattern seen by HPLC. Sequential and combined exoglycosidase digestions revealed a novel α-GalNAc-capped hepta-xyloside which was consistent with the mass of the intact molecule and each of the separate digestion products.

The results indicated that α-GalNAc-capping is not restricted to the core region tetrasaccharide of GAG chains, but it was still consistent with its involvement in the chondroitin sulfate biosynthetic pathway. α-GalNAc does not appear to act as a GAG chain termination signal at least for aggrecan (Plaas *et al.*, 1997) nor as a biosynthetic intermediate for chondroitin sulfate (Kitagawa *et al.*, 1997). Biosynthesis of an extended chondroitin sulfate-like xyloside capped with α-GalNAc is also consistent with recent report of Kitagawa *et al.*, who showed that an α-*N*-acetylgalactosaminyltransferase (α-GalNAcT) in bovine serum catalyzes the transfer of α-GalNAc from UDP-GalNAc to a series of GAG-related synthetic glycoserine molecules (Kitagawa *et al.*, 1995). They also reported that the enzyme transferred GalNAc to a chondro-hexasaccharide serine, GlcAβGalNAcβGlcAβGalβGalβXyl-Ser, less efficiently than to the linkage tetrasaccharide. Based on these findings, it is likely that both α-GalNAc-capped hepta- and pentasaccharides were produced by the same α-GalNAcT. Hence, the terminal α-GalNAc in the present study is probably attached to GlcA through the same linkage, i.e., in α-1,4, although we could not confirm the positional assignment for the hepta α-GalNAc-capped xyloside due to the limited availability of the material.

One might suggest that the occurrence of α-GalNAc-capped xyloside is a nonspecific reaction involved in phase II detoxification of xenobiotic compounds, but we think this is very unlikely. First, intact melanoma M21 cells have a very limited capacity to glucuronidate MU, <0.3 pmol/h/mg protein. This is at least 3 orders of magnitude less than the nmol/h/mg conversion seen for liver microsomes (Gschaidmeier *et al.*, 1995). Second, although there is precedent for the addition of more than one sugar residue to xenobiotic compounds such as bilirubin (Gordon *et al.*, 1984), this usually occurs in those compounds with more than one potential addition site. There is only one report showing that more than one monosaccharide can be added to a single potential

modification site forming a GlcAβ1,2GlcA-structure on nalmefene (Dixon *et al.*, 1989). Third, melanoma cells incubated with MU did not produce any GalNAcα-GlcAβ-MU on the single potential modification site when assayed by methods that could detect 1 pmol of MU product. Thus, α-GalNAcT is not part of a multistep detoxification pathway in melanoma cells. However, crude microsomes and Golgi fractions prepared from these cells can transfer GalNAc to GlcAβ-MU and the GalNAc is found exclusively in α-linkage. The specific activity in the microsomes and Golgi is quite substantial, in the range of that of the first galactosyltransferase used for GAG chain synthesis (GalT-I) measured with an artificial acceptors. We do not know the linkage position of GalNAcαGlcAβ-MU, but further work is in progress to define this activity.

Still there are questions remaining about the significance of α-GalNAc-capped xyloside since this structure has not been reported in naturally occurring glycans. It is important to remember that many glycosyltransferases participate in the addition of similar structures to several different classes of glycans, e.g., poly-*N*-acetyllactosamines which are found on *N*- and *O*-linked oligosaccharides and glycosphingolipids (Fukuda, 1994). It is possible that the addition of α-GalNAc residues to β-GlcA occurs on other glycans besides GAG chains. Identification of an endogenous acceptor for such a modification along with characterization of the enzyme, α-GalNAcT, should reveal the importance of this modification in other glycoconjugates.

Materials and methods

Materials

C18 silica gels were from Analtech, Inc. Silica gel was from Sigma. Human melanoma cells were provided by Dr. J. M. Trent, University of Michigan, Ann Arbor, MI, and the neuroblastoma cell line IMR-32 was obtained from American Type Culture Collection. Tissue culture medium was purchased from Irvine, Inc., and galactose[6-^{3}H] (60 Ci/mmol), UDP-^{3}H-Gal, and UDP-^{3}H-GalNAc were purchased from American Radiolabeled Chemicals, St. Louis, MO. The *Helix pomatia* agglutinin–agarose beads were from EY Laboratories. Bovine testicular β-glucuronidase was kindly provided by Dr. Philip Stahl, Washington University School of Medicine, St. Louis, MO. Human placental β-hexosaminidase A was gift from Dr. Don Mahuran, Hospital for Sick Children, Toronto. *Arthrobacter ureafaciens* neuraminidase, jack bean β-galactosidase, and α-*N*-acetylgalactosaminidase were purchased from Oxford Glycosystems. Xylβ-pNP/MU, Siaα2,6Galβ1,4GlcNAcβ1,3Galβ1,4Glc and Siaα2,3Galβ1,3(Siaα2,6)GlcNAcβ1,3Galβ1,4Glc were from Sigma. GlcAβ-MU was from Calbiochem. Sia-Galβ1,3GlcNAcβ1,3Galβ1,4Glc-AMC was purchased from New England Biolabs, Inc. 2-aminobenzamide (2AB) labeling of Siaα2,6Galβ1,4GlcNAcβ1,3Galβ1,4Glc and Siaα2,3Galβ1,3(Siaα2,6)GlcNAcβ1,3Galβ1,4Glc was performed according to the manufacture's instructions.

Extraction of xylosides from culture medium

Purification of anionic xylosides was performed according to the method described previously (Manzi *et al.*, 1995). For the detection of GlcAβ-MU in culture media, M21 cells were grown in 10% FCS-RPMI 1640 in a 10 cm dish. At 90% confluence, the cells were washed with RPMI 1640 and incubated with 1 mM MU for 24 h. The medium was collected and applied to C18 silica gel column (Analtech). The column was washed with water, and the bound materials were eluted with 50% MeOH. The eluates were dried with a SpeedVac concentrator (Savant). The residue was dissolved in 50 μl of 30% AcOH-DMSO and diluted with 1 ml of acetonitrile. The solution was applied to a silica gel spin column equilibrated with acetonitrile. The column was washed with 400 μl × 3 times acetonitrile, and 400 μl × 3 times 4% water-acetonitrile. Carbohydrate-related compounds were eluted with 50% aqueous acetonitrile, and the eluates were dried. The recovery of sugars was over 98%. The materials were dissolved in 50% aqueous acetonitrile and subjected to amine adsorption HPLC as described below with a fluorescence detector (RAININ) and 0.5 ml fractions were taken. The fractions corresponding to the elution positions of GlcAβ-MU and GalNAcαGlcAβ-MU were pooled, dried and digested with β-glucuronidase, and combination of β-glucuronidase with α-*N*-acetylgalactosaminidase, respectively. The digests were analyzed on C18 reversed phase HPLC to determine the amount of MU formed. This method can detect 1 pmol of MU.

High performance liquid chromatography

Anionic and neutral oligosaccharides were analyzed on an Microsorb-MV column, NH_2, starting with 80% acetonitrile/18 mM ammonium formate, pH 6, for 5 min, followed by a linear gradient of 18–190 mM ammonium formate and of 80–0% acetonitrile at 0.5 ml/min. The radioactivity was monitored using an on-line IN/US βRAM detector. These conditions separate various neutral and anionic oligosaccharides containing one and two negative charges.

Mass spectrometry

FAB-MS analysis was performed at the Scripps Research Institute Mass Spectrometry Facility. MALDI-MS experiments were performed with KOMPACT MALDI I (linear time-of-flight, Kratos Analytical, United Kingdom) mass spectrometer in positive ion mode (nitrogen laser light; λ = 337 nm, pulse; 3 ns). Samples eluted from HPLC analysis was collected, and dried using a SpeedVac. Drying was repeated to remove volatile salt. 0.5 μl (contains 10–100 pmol) of the xylosides dissolved in 50% acetonitrile was applied to a stainless steel target covered with crystalline of a mixture of 2,5-dihydroxy benzoic acid and 1-hydroxy isoquinoline (Mohr *et al.*, 1995) and dried.

HPA affinity chromatography

1 ml of HPA conjugated to agarose beads was packed in a Bio-Spin chromatography Column (Bio-Rad) and equilibrated with 50 mM potassium phosphate buffer, pH 7, containing 150 mM NaCl, 1 mM $CaCl_2$, and 0.02% sodium azide. Samples of about 2000 c.p.m. (^{3}H) and 500 c.p.m. of [^{14}C]fucose as an internal standard in 50 μl of the potassium phosphate buffer were applied to the column, the flow stopped, and incubated for 30 min. The column was then opened and washed with 3 ml of the above buffer followed by elution with 3 ml of 50 mM GalNAc in the same buffer which did not elute any materials. All steps were performed at 4 °C. 300 μl fractions were collected and counted for radioactivity. Recovery of radioactivity was over 96%.

Enzyme digestions

Samples of media extracted by C18 spin column were digested with AUN in 500 μl of reaction mixtures containing 50 mM sodium acetate buffer, pH 5.0, and 10 mU of the enzyme. After heat-inactivation, the digested materials were purified by C18 spin column. For sequential analysis of the xylosides, the samples

were dissolved in 20 µl of reaction mixtures containing 50 mM sodium citrate-phosphate buffer, pH 4.0, and individual or combinations of enzymes; β-glucuronidase (0.3 U), α-*N*-acetylgalactosaminidase (1 mU), galactosidase (1 U), and β-*N*-acetylhexosaminidase A (2 U). Samples for HPLC analysis were diluted with an equal volume of acetonitrile and heated at 100°C for 2 min, followed by centrifugation for 10 min to remove any precipitates.

Compositional analysis

The sample was hydrolyzed in 20% trifluoroacetic acid, 100°C, for 4 h, and the hydrolysate was dried. The residue was dissolved in 80 µl saturated $NaHCO_3$, and 4 µl of acetic anhydride was added and incubated at 22°C for 30 min. The solution was passed through a DOWEX 50 (H^+) spin column, and the run-through and water washes were combined and dried. The residue was labeled with 2-aminobenzamide as described previously (Bigge *et al.*, 1995). The reaction mixture (5 µl) was diluted with 400 µl of acetonitrile, applied on a silica gel spin column which was prewashed with water and then preequilibrated with acetonitrile. The column was washed with 4 × 400 µl of acetonitrile, and 3 × 400 µl of 4% water in acetonitrile. The labeled samples eluted with water were dried and stored at –20°C before analysis on HPLC using C18 reverse phase, Microsorb-MV C18 column (4.6 mm ID × 25 cm), and a mobile phase of 1% acetonitrile, 240 mM ammonium formate, pH 4 (isocractic).

Subcellular fractionation

Subcellular fractionation was performed using a discontinuous sucrose gradient as described previously (Bole *et al.*, 1986). After the centrifugation, ~24 fractions containing 500 µl each were collected from the bottom of the tubes. Each fraction was assayed for endoplasmic reticulum (α-glucosidase II) and Golgi marker enzymes. Protein was estimated by Micro BCA (Pierce) using bovine serum albumin as standard.

Marker enzyme and glycosyltransferase assay

α-Glucosidase II was assayed as described (Mehta *et al.*, 1995). Galactosyltransferase I was assayed as follows; 5 µl of membrane fractions were incubated in a final volume of 20 µl containing 0.1 µCi of UDP-^{3}H-Gal, 50 mM MES, pH 5.5, 150 mM KCl, 15 mM $MnCl_2$, 5 mM Xylβ-pNP, and 0.1 % Triton X-100 for 1 h at 37°C. To the reaction mixture was added 300 µl of 20 mM $NaHCO_3$, and applied to a C18 spin column. After wash with 20 mM $NaHCO_3$, the product was eluted with 50% MeOH. The radioactivity in elution was counted. β1,4-Galactosyltransferase assay was performed as described above in a reaction mixture containing 1 mM GlcNAcβ-Bz, 0.1 µCi of UDP-^{3}H-Gal, 20 mM Hepes, pH 7.4, 10 mM $MnCl_2$, 0.1% Triton X-100. *N*-Acetylgalactosaminyltransferase reaction was performed in a final volume of 20 µl containing 50 mM MES, pH 6.7, 0.5 mM GlcAβ-MU, 0.1 µCi of UDP-^{3}H-GalNAc, 10 mM $MnCl_2$, 10 mM $MgCl_2$, and 0.1% Triton X-100 for 1 h at 37°C.

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Abbreviations

Xylβ-MU, 4-methylumbelliferyl-β-D-xyloside; Xylβ-pNP, *p*-nitrophenyl-β-D-xyloside; GAG, glycosaminoglycan; HPLC, high performance liquid chromatography; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; AUN, *Arthrobacter ureafaciens* neuraminidase; GlcNAcβ-Bz, benzyl β-*N*-acetyl-glucosaminide; GalNAcT, *N*-acetylgalactosaminyltransferase; GalT, galactosyltransferase; GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; Gal, galactose; Sia, *N*-acetylneuraminic acid; GlcAβ-MU, 4-methylumbelliferyl-β-D-glucuronide; AMC, 7-amino methylcoumarin; 2AB, 2-amino benzamide.

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